University of Alberta

Selection of barley grain affects productivity of dairy cows

by

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DEDICATION

This thesis is dedicated to my mom, whose love, patience, and sacrifice will always be an inspiration to me and to the memory of my grandmother, Marieta.

ABSTRACT

This study evaluated the effects of two lots of barley grain cultivars differing in physical and chemical characteristics (Dillon vs. Xena) on ruminal fermentation, ruminal and total tract digestibility, milk production and dry matter intake of dairy cows. Two experiments were conducted; the first evaluated productivity, and the second evaluated ruminal fermentation and digestibility when cultivars were provided at two dietary concentrations. Cows fed Xena or high starch diets had higher propionate concentration in ruminal fluid and total tract starch digestibility, longer duration that pH was below 5.8 and similar intake compared to Dillon or low starch diets respectively. Cow fed Xena also had lower milk fat concentration and higher milk yield compared to cows fed Dillon. This research demonstrated that selection of barley grain can affect milk production, rumen fermentation and total tract nutrient digestibility to an extent at least as great as changes in dietary starch concentration.

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LIST OF ABBREVIATIONS

BCS	Body condition score
BW	Body weight
CM	Corn mix
CP	Crude protein
DHIA	Dairy herd improvement association
DM	Dry matter
DMI	Dry matter intake
EE	Ether extract
FCM	Fat corrected milk
MUN	Milk urea nitrogen
N	Nitrogen
NAN	Non-ammonia nitrogen
NANMN	Non-ammonia non-microbial nitrogen
NEFA	Non-esterified fatty acid
NDF	Neutral detergent fiber
NEL	Net energy of lactation
NSC	Non-structural carbohydrate
OM	Organic matter
PI	Processing index
SCC	Somatic cell count
SD	Standard deviation
TMR	Total mixed ration
TRDOM	Truly ruminally digested organic matter
VFA	Volatile fatty acid

1.0. LITERATURE REVIEW

1.1. Introduction

Carbohydrates comprise 60-70% of dairy cattle ration and are the most important source of energy for rumen microbes. Rumen microbes ferment carbohydrates for energy and the end products of the fermentation are volatile fatty acids **(VFA)**, methane, carbon dioxide and microbial biomass. Volatile fatty acids are absorbed through the rumen wall and utilized by the host animal as an energy source or as a carbon source for the synthesis of glucose, non-essential amino acids or fatty acids. Carbohydrates can be classified into four major categories: 1) Free- monosaccharides and disaccharides; 2) Intracellular- soluble and storage (starches and fructans); 3) Cell wall- cellulose, hemicellulose, pectin, gums and lignins; 4) Chitin- exoskeleton and cell wall (Van Soest, 1994). Free and intracellular carbohydrates together are called non-structural carbohydrates **(NSC)**.

Starch is a storage polysaccharide synthesized by plants, which constitutes approximately 700 g/kg of the dry matter of cereal grains and is the main energy component of ruminant diets (Russell and Gahr, 2000). Starch properties are mainly determined by concentrations of amylose (α1-4 linkages) and amylopectin (α1-4 and α1-6 linkages), degree of crystallinity and level of interaction between starch and protein (Rooney and Pflugfelder, 1986; Van Soest, 1994). According to Rooney and Pflugfelder (1986) many factors can affect starch digestibility: 1) composition and physical form of the starch, 2) protein-starch interaction, 3) cellular integrity of the starch-containing unit, 4) anti-nutritional factors (eg, phytate, tannins and lectins), 5) physical form of the feed, 6) amylose concentration, 7) crystillinazation (as in steam-flaking), and 8) size of starch granules. Several enzymes are required to degrade starch to glucose. Starch is first hydrolyzed by α-amylase yielding maltose, maltotriose, and α-limit dextrins (Alpers, 1994). Disaccharidases hydrolyze the starch

breakdown products, and once free glucose is formed it will either be absorbed or used as a fermentation substrate.

Variable amounts of starch reach the small intestine when high starch diets are fed. Post ruminal starch digestion yields free glucose and is very similar to monogastric intestinal starch digestion, where starch is digested by pancreatic and gastrointestinal enzymes. Owens et al. (1986) suggested that rumen by-pass starch yields 42% more energy than starch digested in the rumen, since energy is not lost by fermentation.

Excess or overly rapid ruminal starch fermentation can lead to ruminal acidosis. When rumen papillae are not able to absorb all the VFA produced from readily fermented carbohydrates and ruminal buffering capacity is not sufficient to neutralize fermentation acids, ruminal pH is decreased. Rumen acidosis can increase the incidence of bloat and laminitis (Orskov, 1986), decrease DMI (Allen, 2000), microbial protein production (Oba and Allen, 2003c) and fiber digestibility in the rumen (Grant and Mertens, 1992).

Lactating dairy cattle have a high energy demand to maintain high milk production and to meet such requirements an energy dense ration is necessary. Barley is the primary cereal grain for feeding dairy cattle in Alberta. In 2001 dairy cattle in Alberta consumed 225,000 tonnes of barley and beef cattle consumed 2.7 million tonnes (Statistics Canada, 2001). Because of greater barley use by beef cattle, much of the research evaluating barley feed efficiency has focused on beef cattle. By contrast, research evaluating the effect of barley-based diets on performance of dairy cattle has mainly compared differences between corn and barley based diets overlooking barley cultivar differences. The morphological, chemical and physical composition of barley cultivars are variable, therefore tabular values of feed composition do not always accurately represent barley grain fed to

animals. Over 50 barley cultivars differing in nutrient composition are grown in Alberta and research has shown cultivars can affect swine and beef cattle productivity. However no research has evaluated the effect of barley cultivar on the productivity of lactating dairy cows.

1.2. Starch Digestion

1.2.1. Ruminal starch digestion

The rumen is the primary site of carbohydrate digestion. Rumen microorganisms digest 60-90% of starch, depending on type of processing and species of grain (McAllister and Cheng, 1996). Bacteria are the main starch digesters, followed by protozoa (Huntington, 1997). The ruminal microbial population is highly dependent on the diet; in high concentrate diets amylolytic bacteria will thrive and in high forage diets cellulolytic bacteria will thrive (Van Soest, 1994).

Microbial attachment is the first step of ruminal starch digestion. Microbes attach themselves to the surface of the substrate by means of a glycoprotein matrix between the cell wall of the substrate and the cell wall of the microbes. Microbes then secrete enzymes into the space enclosed by the glycoprotein matrix and substrate is hydrolyzed (Russell and Gahr, 2000). McAllister et al. (1994) concluded the hyphae of fungi help in bacterial attachment. After attachment amylases, amyloglucosidase and other enzymes are secreted and the substrate is hydrolyzed. Rumen protozoa decrease starch hydrolysis in the rumen as they ingest starch granules and prey on starch digesting bacteria (Kotarski et al., 1992).

After the starch is hydrolyzed, its component glucose is transported across the microbial plasma membrane by an active system (Russell and Gahr, 2000). Once inside the microbe, the glucose is metabolized to pyruvate in the Embden-Meyerhof-Parnas glycolytic pathway. VFA are then synthesized from pyruvate. The

three main VFA found in the rumen are propionate, acetate and butyrate. Propionate is produced by two pathways: the randomizing (high forage diets) and acrylate pathways (high grain diets). Acetate is synthesized from an acetyl-CoA intermediate. VFA exit the cell by diffusion and permeate into the fluid environment of the rumen. The rumen epithelium has the potential to absorb all the end-products of fermentation. While some are passively diffused in an undissociated form, anionic uptake predominates, which is coupled with the exchange of bicarbonate for luminal anions.

An active glucose sodium-dependent transporter, SGLT-1, is found in the rumen epithelium (Aschenbach et al., 2000). However, rumen microbes take up glucose very efficiently, causing the rumen concentration of glucose to be low (< 0.7 mmol/L; Kajikawa et al., 1997). A study done by Aschenbach et al. (2000) showed that even though SGLT-1 in the rumen epithelium has high affinity for glucose (Michaelis-Menten constant $K_{0.5}$= 0.28 mmol) under normal conditions glucose disappearance in the rumen is equivalent to <0.1% of the daily metabolizable energy intake. When intraruminal glucose concentration is high (12 mmol/L), glucose uptake by SGLT-1 is approximately 4% of the daily metabolizable energy intake (Aschenbach et al., 2000). The same group hypothesized that the sodium-coupled absorption of glucose is a form of defence mechanism against the start of ruminal acidosis. This theory is supported by a high clearance in luminal glucose concentrations, even in animals not adapted to high starch rations, however long term effects of high starch rations on sodium-coupled absorption of glucose remains unknown (Aschenbach et al., 2000).

Microbes must overcome two main obstacles in order to digest starch, one is the seed coat and the other is crystallization of starch in the kernel (Harmon and Taylor, 2005). In order to permit starch digestion the seed husk must be damaged.

When starch is not gelatinized during processing, the starch granule structure may limit extent of digestion (Tester et al., 1995; Tester et al., 1991). Amylose and amylopectin are packed in semi-crystalline granules and organized in layers (Harmon and McLeod, 2001) and are surrounded by a protein matrix. The protein matrix associated with starch granules affects its digestibility. The disruption of the protein matrix is essential in improving digestion for some grains. McAllister et al. (1993) found that the protein matrix of corn limits the access of rumen bacteria to starch granules and that protein and structural carbohydrate within the cereal kernel could be more important in determining ruminal starch digestion than the chemical and physical form of starch.

Starch degradation rates are greatly affected by grain processing methods. When grain is processed by the combination of heat, moisture, mechanical action and time, the pericarp is damaged and bacteria can attach to starch and digest it. Ruminal digestion of starch is slowed down and limited when the feed is treated with formaldehyde or phenolics (Fluharty and Loerch, 1989; Mahmoudzadeh et al., 1985; McAllister et al., 1990; Oke et al., 1991; McAllister et al., 1992a; McAllister et al., 1992b; Castlebury and Preston, 1993). With the reduction in rate of ruminal digestion, the amount of protein and starch reaching the small intestine increases.

1.2.2. Intestinal starch digestion

Usually the amount of starch entering the small intestine is negligible when high forage diets are fed, but when diets with high levels of cereal grain are fed, significant quantities of starch can enter the duodenum. Pre-digestion of starch in the rumen influences the quantity and composition of starch reaching the small intestine (Owens et al., 1986). Most of the post ruminal starch digestion (5-20% of total starch consumed) occurs in the small intestine (Streeter et al., 1989; Hill et al., 1991;

Streeter et al., 1991; Zinn, 1991). Harmon and Taylor (2005) found that 35-60% of starch entering the small intestine is degraded there and 35-50% of the starch escaped is degraded in the large intestine. It was shown that bacteria and protozoa can contain up to 26-38% of their mass in polysaccharides, contributing to the starch reaching small intestine (Hespell and Bryant, 1979). When only forages are fed, microbial polysaccharides account for all starch flow to the small intestine, however when high concentrate diets are fed some dietary starch escapes rumen fermentation (Owens et al., 1986).

The pancreas and the intestinal mucosa both synthesize amylase used in starch hydrolyses in the small intestine. According to Owens et al. (1986), starch digestion to glucose requires amylase, which hydrolyzes amylose and amylopectin, maltase to cleave released maltose, and isomaltase to cleave the branch points of amylopectin. Glucose can then be absorbed by the epithelial cells lining the small intestine (Van Soest, 1994). Intestinal mucosa amylase hydrolyzes starch faster than pancreatic amylase, due to the fact that intestinal mucosa amylase aids in transfer of released maltose to the maltase tightly bound to the brush border, which enhances glucose absorption (Owens et al., 1986).

D-glucose and D-galactose are transported from the lumen to the enterocytes with the aid of the SGLT-1 protein (Shirazi-Beechey et al., 1991). The abundance and activity of SGLT-1 protein in new born ruminants is high, however the number and activity tends to decrease after weaning. However, Huntington et al. (2006) disagree that SGLT-1 is responsible for glucose transport from the intestinal lumen into the portal circulation because no adaptive response to luminal substrate is observed in cattle. Sugars can cross the luminal membrane in the facilitated transporter GLUT5 which transports fructose and GLUT2 which transports glucose

and fructose, but both facilitated transporters have not been characterized in ruminants (Harmon and Taylor, 2005).

Starch hydrolysis in the small intestine is variable and there are several hypotheses to explain these variations. Harmon (1992) reported that starch digestibility in the small intestine varied from 10-93% for beef steers and Russell et al. (1986) found that glucose absorption in dairy steers ranged from 10-100%. Possible causes for variations may be inadequate enzyme production, excretion and activity, limitations in glucose absorption, rate of starch passage, insufficient enzyme attachment to starch (Owens et al., 1986), suboptimal pH for enzyme activity (Russell et al., 1981) and physicochemical characteristics of grain particles (Oba and Allen 2003a,b). Huntington (1997) suggested that the limitation of pancreatic amylase is the reason why starch is not completely digested in the small intestine. Many factors are known to limit the activity of pancreatic amylase. An increase in DMI will not lead to an increased production of pancreatic amylase, however an increase in energy intake will lead to an increase in pancreatic amylase secretion (Harmon, 1993). Russell et al. (1981) demonstrated that the optimum pH for pancreatic amylase is 6.9, and as the pH increases or decreases 0.5 pH units from 6.9 pancreatic amylase activity is decreased by 20%. On the other hand the optimum pH for pancreatic maltase is 5.8 (Russell et al., 1981). Mahmoudzadeh et al. (1985) suggested tannins and lignin derivatives can reduce amylase activity. Chittenden et al. (1984) infused glucose, maltose and starch in the small intestine to manipulate amylase secretion and concluded that glucose infusion increased pancreatic amylase at d 16 but not at d 23, maltose infusion showed no difference and starch infusion decreased pancreatic amylase both days. According to Harmon and Taylor (2005) mucosal enzymes or glucose transporters may be limiting starch digestion and absorption in the small intestine and not necessarily amylolytic enzymes.

However, Kreikemeier (1991) showed that glucose absorption is not the main limiting factor in starch digestion. When 60 g/h of glucose were abomasally infused 94% of small intestine glucose disappearance was accounted for as net glucose portal absorption but when starch was infused this percentage dropped to 43%, indicating that glucose absorption is not be the primary limiting factor of starch digestion.

Starch reaching the large intestine can be hydrolyzed. As starch flow to the duodenum increases, greater amounts of starch reach the large intestine, however this is undesirable since the large intestine has lower starch digestion efficiency compared with the small intestine (Harmon and McLeod, 2001). Starch digestion in the large intestine is similar to ruminal starch digestion, with VFA, methane, carbon dioxide and microbial biomass as end-products (Armstrong and Smithard, 1979). While VFA can be absorbed and utilized, microbial protein is not absorbed and is excreted in feces (Armstrong and Smithard, 1979).

1.2.3. Effects of starch digestion on post-absorptive energy metabolism

Ruminants rely on gluconeogenesis to meet their glucose requirements. Twenty-five percent or less of their glucose supply comes from starch digestion into glucose in the gut (Huntington, 1997). The primary source of carbon for gluconeogenesis is propionate, and gluconeogenesis is necessary to maintain glucose homeostasis and its utilization for maintenance and growth (Leat, 1970). As starch digestion shifts from the rumen to the small intestine glucose absorption by the portal-drained viscera increases (Taniguchi et al., 1995). When the concentration of plasma glucose increases insulin is secreted by the pancreas. Eisemann and Huntington (1994) studied the effect of insulin on liver glucose and concluded that an increase in insulin release causes a decrease in glucose production from propionate, in other words insulin controls gluconeogenesis. While the removal of propionate

from the blood is a metabolic priority (Huntington and Richards, 2005), propionate will not be converted into glucose at elevated insulin levels; rather it will be oxidized in the liver. Other glucose precursors are L-lactate and glucogenic amino acids. L-Lactate, like propionate, is an end-product of fermentation and amino acids are primarily from dietary protein that escapes the rumen, microbial protein and a small portion comes from the Cori cycle and deamination and transamination of glucogenic amino acids (Huntington and Richards, 2005).

Energetic efficiency is greater if starch is hydrolyzed and absorbed in the small intestine rather than in the rumen. Only 75% of energy from carbohydrate fermentation is retained as VFA. The complete oxidation of propionate to glucose only yields 65% of the energy that would be available if carbohydrate was digested and absorbed directly as glucose. However without the presence of the microbes ruminants would not be able to digest fibre, the primary dietary component for ruminants, so overall the efficiency of ruminant digestion is a compromise.

Glucose is used by almost all tissues for the oxidative metabolism yielding ATP (Ortigues-Marty et al., 2003). When glucose availability increases due to greater postruminal starch digestion, total glucose irreversible loss increased in lactating cows (Knowlton et al., 1998). Huntington and Richards (2005) suggested that the amount of dietary starch that is required to support glucose irreversible loss increases as more starch is degraded in the rumen, mainly due to inefficiencies of fermentation.

1.2.4. Effects of starch digestion in microbial protein synthesis

Microbial protein is the primary source of metabolized protein for ruminants. The amino acid profile of microbial protein closely resembles ruminant requirements and therefore to maximize production it is vital to maximize microbial protein

production (O'Connor et al., 1993). Many factors influence microbial protein synthesis: 1) availability of substrates, 2) energy supplied form organic matter for rumen fermentation, 3) energy spilling or uncoupling reactions (Strobel and Russell, 1986), 4) rate of passage and 5) rumen pH.

If carbohydrate availability is insufficient to match protein degradation in the rumen, nitrogen can be lost as ammonia. Some microbes can incorporate amino acids from the diet into microbial protein if sufficient energy is available, otherwise the microbes ferment them for energy, which releases ammonia in the rumen (Nocek and Russell, 1988). High ammonia concentration in the rumen can lead to absorption of ammonia into the blood stream, which at high levels is very toxic to the animal and very costly for the liver to detoxify to urea. Taniguchi et al. (1995) found that the synchronization of starch and nitrogen supply in the rumen increased nitrogen retention and decreased ammonia absorption in steers. Ruminal outflow of microbial protein is increased when readily available energy from starch is available (Spicer et al., 1986; Streeter et al., 1991; Poore et al., 1993; Zinn, 1993a, b, 1988). For example, Poore et al. (1993) found that ruminal outflow of microbial protein was increased from 2.2 to 2.8 kg/d when dairy cows where fed steam-flaked versus dry-rolled sorghum. Their observation reinforces the impact of starch availability in the rumen on microbial protein production.

Protein solubility greatly affects the rate of fermentation in the rumen. According to Nocek and Russell (1988) the rate of protein and carbohydrate fermentation in the rumen is controlled by: 1) the intrinsic rate of protein hydrolysis, 2) uptake rate of peptides and amino acids into microorganisms, 3) availability of carbohydrate to provide ATP for microbial protein synthesis, and 4) presence of methanogenic bacteria to provide a hydrogen sink. Chamberlain et al. (1985) suggested that the source of carbohydrate greatly influences the concentrations of

ruminal ammonia by changing the microbial population in the rumen. Maximizing starch concentration in the diet does not mean maximizing microbial protein synthesis. Foresburg et al. (1984) performed in vitro experiments and found increased microbial synthesis from high starch diets, however the optimal amount of starch for dairy cattle is not the maximal amount (De Visser and de Groot, 1980; MacGregor et al., 1983). For example, Aldrich et al. (1993) reported that diets containing 36% NSC resulted in the highest passage of microbial nitrogen to the duodenum. The optimum starch concentration in dairy cow diets is that which improves milk production by promoting maximum microbial protein synthesis.

1.3. Barley grain

1.3.1. Environmental effects on grain growth

Barley grain is usually produced in climates that are inappropriate for maize or rice production (Poehlman, 1985). Barley crops thrive in cool dry climates and perform poorly in hot humid climates (Beaven, 1947). At elevated temperatures sucrose synthase activity (ie., the enzyme that cleaves sucrose) is reduced leading to lower barley starch content, decreased endosperm volume and space available for starch deposition (Duffus and Cochrane, 1993). Compared to other cereal grain barley can mature at higher latitudes such as mountain slopes (Nuttonson, 1957). Sporadic periods of drought can lead to premature ripening, high nitrogen levels and thin steely kernels (Poehlman, 1985). Barley can be grown in a wide range of photoperiods and is considered a drought resistant crop (Guitard, 1960).

Barley crops will flourish in a well-drained fertile loam or light clay soil (Morgan et al., 1938) and on soil pH ranging from 6.0-8.5 (Poehlman, 1985). The yield of barley tends to increase with nitrogen application but the hordein content is also increased. An increase in hordein lowers protein quality (Duffus and Cochrane,

1993) as it is a prolamine protein low in lysine. It was seen in studies with pigs (Newman et al., 1978 and Newman et al., 1977) and rats (Doll et al., 1974; Munck, 1972, and Newman et al., 1974) that high lysine barley cultivars increased nutritional protein quality.

1.3.2. Nutrient composition

The nutrient composition of barley grain varies among cultivars and is highly affected by growing conditions. Starch concentration is greatly influenced by environmental aspects but the amylopectin amylose ratio can be altered by genetic selection (Kasha et al., 1993). The kernel, which is primary starch, is the main source of energy, even though it is low in oil content (Newman and McGuire, 1985). The levels of CP in the grain vary from 7.5 to 17% (LaFrance and Watts, 1986), NDF from 17.3-32.1% (Ovenell-Roy et al., 1998a) and starch from 45.9-62.8% (Ovenell-Roy et al., 1998a).

Herrera-Saldana et al. (1990) evaluated the rates of starch digestion for five cereal grains *in situ* and *in vitro*. When grains were processed similarly oats had the fastest rate of starch digestion, followed by wheat, then barley, then corn, and finally sorghum (Table 1.1; Herrera-Saldana et al., 1990). Spicer et al. (1986) compared diets containing corn, barley or sorghum (82% grain). Corn ruminal starch digestion (83.7%) and barley ruminal starch digestion (87.7%) were significantly higher than that of sorghum (72.5%). Other studies also found barley to have higher ruminal starch digestibility than corn and sorghum. McCarthy et al. (1989) reported the ruminal starch digestibility for barley to be 77.4% and ground corn to be 48.6% and Herrera-Saldana and Huber (1989) determined barley ruminal starch digestibility to be 80% and sorghum ruminal starch digestibility to be 49%.

1.3.3. Barley vs. corn

Numerous experiments have evaluated the effect of substituting corn for barley in dairy rations. Casper et al. (1990), McCarthy et al. (1989), and Overton et al. (1995) found that milk yield was decreased when cows were fed barley instead of corn. However other research observed no treatment effect on milk yield (Grings et al., 1992; Khorasani et al., 1994). In terms of milk composition, Casper et al. (1990) reported milk fat increased when corn replaced barley, but other researchers (Bilodeau et al., 1989; Casper and Schingoethe, 1989; De Peters and Taylor, 1985; McCarthy et al., 1989) found no differences in milk fat content. Milk lactose and protein were not different between barley and corn based diets in previous studies (De Peters and Taylor, 1985; Bilodeau et al., 1989; McCarthy et al., 1989, Grings et al., 1992).

Rapidly fermented starch sources often depress DMI. Decreased milk production for cows fed barley compared to corn grain was generally attributed to decreased DMI. Dry matter intake was higher for cows fed corn instead of barley (Casper and Schingoethe, 1989; McCarthy et al., 1989; Casper et al., 1990; Khorasani et al., 1994; Yang et al., 1997a;b). However other studies have not observed low intake of cows fed barley diets (De Peters and Taylor, 1985; Grings et al., 1992, Casper et al., 1999). Inconsistent effects of grain source on DMI can be attributed to the proportion of grain in the diet (DeVisser et al., 1990; Grings et al., 1992), the starch concentration of the diet (Tommervik and Waldern, 1969; Rode and Satter, 1988; DeVisser et al., 1990), and forage particle length (Rode and Satter, 1988). Diets containing higher concentrations of forage NDF promote chewing, salivation, and high rumen pH (Mertens, 1997), which mask some effects caused by differences in fermentability of diets in the rumen.

1.3.4. Effects of barley grain processing on productivity

Barley processing is necessary since kernels are hardly affected by mastication (Beauchemin et al., 1994). When whole kernels were fed to steers, 48.2% of all whole kernels were recovered in the feces (Toland, 1976). Physical processing of barley increases digestibility of grain by disruption of the pericarp. Ruminal and post ruminal starch digestion tend to be increased when the processing index of barley is reduced. Processing index **(PI)** is defined as the volume weight of barley after processing, expressed as a percent of its volume weight before processing (Yang et al., 2000). Beauchemin et al. (2001) evaluated the effect of barley tempered to four extents (PI of 82, 75, 70 and 65%) on finishing steers, and reported that DMI was not affected by the extent of processing, however total tract starch digestibility increased with greater extent of processing while CP digestibility was not affected by treatment. When dry-rolled barley (density of 0.39 kg/L) or steam-rolled barley at two densities (0.39 and 0.19 kg/L) were fed to steers in a 74.3% barley finishing diet, average daily gain was similar but feed intake was lower for steam-rolled barley than dry-rolled barley (Zinn, 1993a). Dry-rolled barley had lower total tract digestibility for OM and starch than steam rolled-barley (Zinn, 1993a). However, ruminal and total tract digestion of OM and starch was similar for both steam-rolled barleys and there was an increased passage of non ammonia-nitrogen with low density steam-rolled barley compared with high density steam barley (Zinn, 1993a).

In a study to evaluate the effect of barley grain processing thickness, 1.60 mm and 1.36 mm, dry matter intake was increased with decreasing thickness because of increased extent of digestion (Yang et al., 2001). Average rumen pH was lower for cows fed 1.36 mm barley, but the digestibility of DM, OM, NDF and starch was higher for 1.36 mm than 1.60 mm barley. Milk yield and milk protein was higher

for cows fed 1.36 mm barley but milk fat was lower (Yang et al., 2001). The effects of processing barley at four different PI (81.0, 72.5, 64.0, and 55.5%) was evaluated for lactating dairy cows (Yang et al., 2000). An increase in DMI was observed with a decrease in PI with maximum intake at the PI of 64.0%. Intestinal and total tract digestibility increased with decreasing PI. Milk yield increased with decreasing PI with the highest production at the PI of 64% (Yang et al., 2000). Barley processing is essential to maximize utilization by cattle, it was concluded that animal performance was increased when barley was steam rolled compared to dry rolled, when processing thickness was decreased and PI was 64%.

1.3.5. Effects of barley grain cultivar on productivity

1.3.5.1. Dairy Cattle

While barley is the primary grain source fed to ruminants in Western Canada, not much research has been done evaluating the effect of barley cultivar on productivity of lactating dairy cows. Yang et al. (1997a,b) fed hull-less barley and hulled barley to dairy cows and found no difference in milk production even though cows fed hulled barley had higher ruminal and postruminal digestibility.

1.3.5.2. Beef Cattle

The effect of barley cultivar on beef cattle performance has been evaluated in numerous studies. Differences in the feeding value of barley can be attributed to cultivar differences, growing environment, processing method and kernel morphology (Ovenell et al., 1993). Bradshaw et al. (1996) fed two barley cultivars with the same test weight (67 kg/hl), Steptoe (six-row feed cultivar) and Klages (two-row malting cultivar)) to growing (31% barley) and finishing steers (87% barley) and found no differences in steer performance. However when six studies were performed over

five years Klages increased weight gain once, decreased weight gain twice and no difference was seen for weight gain for the remaining three studies (Krecher et al., 1986). Ovenell-Roy et al. (1998a,b) fed six barley cultivars to steers in finishing diets and found that cultivars with lower NDF content had lower digestibility of other nutrients. It was also concluded that in this study two-row cultivars had a higher feeding value than six-row cultivars.

Covered (Leduc, 61.9 kg/hl) and hull-less (Condor, 76.1 kg/hl) barley was fed to steers in finishing diets (77% barley) and gain was found to be similar for both cultivars, however Leduc fed animals had higher intake, leading to a decreased feed efficiency and dietary energy density (Zinn et al., 1996). A study evaluating three cultivars, Gunhilde (two-row, 64.5 kg/hl), Harrington (two-row malting, 63.1 kg/hl) and Medallion (six-row, 61.9 kg/hl), concluded that Medallion had the better feed conversion when fed to steers in finishing diets (80% barley) followed by Gunhilde and Harrington (Boss and Bowman, 1996a). When starch digestion was evaluated for Gunhilde, Harrington and Medallion, no significant differences were observed (Boss and Bowman, 1996b). Effects of test weight on productivity of beef cattle have not been consistent.

Hinman (1978) evaluated barley with different test weights (54.1, 57.9, 63.0 and 65.6 kg/hl) and found that animal performance increased with increasing test weight. Barley quality should not be based on cultivar alone, since growing environment can greatly affect feeding value. Grimson et al. (1987) examined barley grain of three test weights (47.7, 55.4 and 67.0 kg/hl) and two processing methods (steam flaked and dry rolled) and found no difference in daily gain or dry matter intake, however feed efficiency for the 47.7 kg/hl barley was poorer than 55.4 and 67.0 kg/hl. Mathison et al. (1991) suggested there was no marginal benefit for greater test weight once it exceeds 58.9 kg/hl.

1.4. Conclusion

The nutrient composition of barley grain is affected by many factors. Growing conditions and location, genetic variation, and physical characteristics influence the nutrient composition of barley grain. There is limited research which evaluated the effect of barley with different nutrient on dairy cows. Therefore the objectives of this study were to evaluate the effect of barley grain with different chemical composition and physical characteristics on DMI, productivity and ruminal fermentation of lactating dairy cows.

Table 1. 1. Starch content, degradability (in vitro) and rate of degradation (in vitro) of five cereal grains (Herrera-Saldana et al., 1990)

Item	Corn	Sorghum	Wheat	Barley	Oats
Starch content, % DM	75.7	71.3	70.3	64.3	58.1
Time (m)			(% total starch digested)		
0	7.7	2.9	1.7	10.6	3.9
15	8.5	7	6.8	11.4	16.5
30	9.4	7.8	12.7	13.8	19.9
45	11.5	8.5	19.3	15.5	23.1
60	13.1	9.4	24.2	18.1	28
Degradation rate, $\%/h^2$	6.4	3.1	23.5	8.8	15.1

1.5. References

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2.0. EFFECT OF GRAINS DIFFERING IN EXPECTED RUMINAL FERMENTABILITY ON PRODUCTIVITY OF LACTATING DAIRY COWS*

2.1. Introduction

Barley is the primary grain fed to ruminants in Northwestern US, Western Canada, and Europe. Barley is highly degradable in the rumen and often causes excess fermentation, acid production, decreasing rumen pH (McCarthy et al., 1989; Robinson and Kennelly, 1989; Nocek and Tamminga, 1991). Overton et al. (1995) fed dairy cows diets containing five different ratios of starch from ground shelled corn and steam-rolled barley and found that DMI and milk yield decreased as the ratio of starch from steam-rolled barley increased. Rapid rate of carbohydrate degradation is often associated with sharp reductions in DMI and milk production (McCarthy et al., 1989; Aldrich et al., 1993; Mitzner et al., 1994). Thus, the decreased DMI and productivity for cows fed barley grain compared with corn grain can be in part attributed to excess fermentation in the rumen.

A significant variation exists in chemical composition of barley grain. It is reported that barley grain varies from 7.5 to 15% in CP concentration (LaFrance and Watts, 1986), from 17 to 32% in NDF concentration (Ovenell-Roy et al., 1998b), and from 45.9 to 62.8% in starch concentration (Ovenell-Roy et al., 1998b). Furthermore, Khorasani et al. (2000) reported that the rate of in situ DM degradation varies from 20.0 to 64.4 %/h. Yu et al. (2004) showed distinctive differences in chemical and structural endosperm matrix makeup between barley cultivars Valier and Harrington using infrared microspectroscopy, and these differences are expected to affect rate of starch degradation in the rumen.

Barley grain differing in chemical or physical characteristics is expected to affect productivity of animals. For example, barley cultivars varying in nutrient

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composition affect feed efficiency of beef cattle (Boss and Bowman, 1996; Zinn et al., 1996). However, little research has been conducted to evaluate the effects of barley grain cultivars on productivity of lactating dairy cows. We hypothesized that barley grain varying in physical and chemical characteristics would affect productivity of lactating dairy cows. Further, because excess fermentation in the rumen often decreases DMI (Allen, 2000), we also hypothesized that DMI and milk production of cows fed a less fermentable source of barley would be similar to cows fed corn. Therefore, the objective of this study was to evaluate the effect of grains differing in starch content and expected fermentability in the rumen on DMI and productivity of lactating dairy cows.

2.2. Material and Methods

2.2.1. Cows, Diets and Treatments

Twenty-two multiparous and nine primiparous Holsteins cows in early to midlactation (94 ± 29 DIM; $\overline{X}$ ± SD) from the University of Alberta Dairy Research and Technology Center were randomly assigned to a treatment sequence within a 3 × 3 Latin square balanced for carry over effects. At the beginning of the experiment cows averaged 626 ± 84 kg and BCS was 3.0 ± 0.35. Treatment periods were 21 d with the final 7 d used to collect samples and data. Cows were cared according to guidelines of the Canadian Council on Animal Care (Institutional Animal Use Approval Number: 2005-13C).

Two 15-t lots of barley grain, cultivars Xena and Dillon, were obtained from a local grain company. These two lots were selected because of the distinctive differences in their physical and chemical characteristics (Table 2. 1). A corn mixture (CM) containing 87.5% dry ground corn, 11.4% beet pulp, and 1.1% urea on a DM

basis was prepared. The CM was designed to approximate CP and starch concentrations of barley grains. Starch concentration was 50.0, 58.7, and 60.4% and 6-h in vitro starch digestibility was 73.5, 78.0 and 71.0%, respectively for Dillon, Xena and CM. Although actual ruminal fermentability of grains was not measured in this study, it is expected to differ because of the differences in starch concentration and 6-h in vitro starch digestibility. Three experimental diets were formulated to contain approximately equal amounts of steam-rolled barley, using either Dillon or Xena, or CM (Table 2. 2). All diets were designed to meet or exceed requirements for CP, RUP, vitamins, and minerals (NRC, 2001). The diets were formulated to contain 19.4% CP and 25.3% forage NDF concentrations. Throughout the experiment, cows were housed in tie stalls with continuous access to water. The experimental TMR was offered once daily (0900 h) at 110% of expected intake and cows were milked in their stall twice daily (0600 and 1700 h). Animals were allowed to exercise once daily (1100 h) for 2 h.

2.2.2. Data and Sample Collection

Body weight was recorded on two consecutive days immediately prior to the start of the first period and on the last 2 d of each period. Body condition score was determined by two trained individuals at the beginning of the experiment and at the end of each period (five-point scale was used with 1= thin and 5 = fat; Wildman et al., 1982). The amount of feed offered and orts were weighed and recorded daily during the collection period. Representative samples of all dietary ingredients, TMR, and orts (approximately 12.5%, as-fed basis) were collected daily during the collection period. The orts were pooled and one sample was retained per cow per period. The DM content of barley silage and alfalfa hay was determined weekly to adjust dietary allocation of forages to maintain consistent forage to concentrate ratio. Milk yield was

measured daily and averaged over the collection period. Milk was sampled at every milking on d 19, 20 and 21 of each period and milk component concentrations were averaged for these dates. Fecal and blood samples were collected from each cow on d 15-21 every 28 h to account for diurnal variations (11 AM on d 15, 3 PM on d 16, 7 PM on d 17, 11 PM on d 18, 3 AM on d 20 and 7 AM on d 21). Fecal samples (150g) were collected from the rectum and frozen at -20°C, and composited into one sample per cow per period immediately prior to drying in a forced air oven. Blood was sampled from the coccygeal vessels using vacuatiner® tubes containing sodium heparin (Becton Dickinson, Franklin Lakes, NJ). Blood samples were immediately placed on ice and centrifuged within 1 h at 4°C for 30 min at 3000 × *g*. Plasma was harvested and stored at –20°C until further analysis.

2.2.3. Sample Analysis

Diet ingredients, orts and feces were dried in a 55°C forced air oven for 72 h to determine DM concentration. The dried samples were ground through a 1-mm screen using a Wiley mill (Thomas-Wiley, Philadelphia, PA). Samples were analyzed for concentrations of DM, ash, NDF, indigestible NDF, CP, ether extract (EE) and starch. The DM concentration was determined after drying samples at 135°C for 2 h. Ash concentration was determined after 5 h at 550°C in a furnace. The NDF concentration was determined using amylase and sodium sulfite (Van Soest et al., 1991). Crude protein concentration was quantified by flash combustion with gas chromatography and thermal conductivity detection (Carlo Erba Instruments, Milan, Italy; Handbook of Food Analytical Chemistry, 2005). Starch was measured by an enzymatic method (Karkalas, 1985) after samples were gelatinized with sodium hydroxide, with glucose concentration measured using a glucose oxidase/peroxidase enzyme (Sigma, No. P7119) and dianisidine dihydrochloride (Sigma, No. F5803).

Absorbance was determined with a plate reader (SpectraMax 190, Molecular Devices Corp., Sunnyvale, CA). Ether extract concentration was determined using a Goldfisch extraction apparatus (Labconco, Kansas City, MO, USA; Handbook of Food Analytical Chemistry, 2005). Indigestible NDF was determined as NDF residue after 120 h incubation in the rumen, and used as an internal marker to determine apparent total tract nutrient digestibility (Cochran et al., 1986).

The TMR and orts samples were analyzed for particle size distribution using Penn State Forage Particle Separator (NASCO, Fort Atkinson, WI) to determine extent of sorting, which was expressed as a sorting index. Sorting index was calculated as the actual intake / expected intake for each portion retained on the individual screens. Expected intake was calculated as the particle size distribution of the TMR (%) × actual as-fed intake. Actual intake was calculated as the amount of feed offered × particle size distribution in the TMR (%) – the amount feed refused × the particle size distribution in the orts samples (%). A sorting index of 1 indicates no sorting, a sorting index of less than 1 indicates sorting against, and greater than 1 indicates sorting for.

Milk fat, protein, lactose and SCC concentrations were measured with infrared spectroscopy by Edmonton-Alberta DHIA (Milk O Scan 605), milk urea nitrogen was determined with an automated IR Fossomatic 400 Milk Analyzer (Foss North America, Brampton, Ontario). Milk energy output was calculated according to NRC (2001) using measured concentrations of milk fat, protein, and lactose. Commercial kits were used to determine plasma concentration of insulin (Coat-A-Count, Diagnostic Products Corporation, Los Angeles, CA) and non-esterified fatty acid (NEFA; NEFA C-kit; Wako Chemicals USA, Richmond, VA) using a previously described protocol modification (Johnson and Peters, 1993). Plasma glucose

concentration was measured using a glucose oxidase/peroxidase enzyme and dianisidine dihydrochloride as described above.

2.2.4. Statistical Analysis

All data were analyzed using the fit model procedure of JMP (version 5.1, SAS Inc., Cary, NC) according to the following model:

$Y_{ijkl} = \mu + C_i + P_j + T_k + e_{ijkl}$

where μ = overall mean

C_i = random effect of cow (i=1 to 31)

P_j = fixed effect of period (j=1 to 3)

T_k = fixed effect of treatment (k= 1 to 3)

e_{ijkl} = residual, assumed to be normally distributed.

Parity × treatment interaction had been originally included in the model, but it was removed because the interaction was not significant for all response variables. Orthogonal contrasts were made to evaluate effect of CM vs. barley (mean of Dillon and Xena) and effect of Dillon vs. Xena.

2.3. Results

The diets contained about 39% barley grains or 40.6% CM. Because the DM concentration of CM ingredient samples collected during the study was greater than expected, the diet actually contained 40.6% rather than 39% CM as intended (DM basis; Table 2. 2). The grain sources were added to the diet in approximately equal proportions, thus the starch content of the diet was highest for CM, intermediate for Xena, and lowest for Dillon.

Cows fed CM had greater DMI compared to cows fed barley grain (23.6 vs. 21.6 kg/d; $P < 0.001$; Table 2. 3). The DMI was not affected by barley cultivar (21.9

vs. 21.4 kg/d; $P = 0.35$). Cows fed CM sorted for longer particles (i.e., feed on the top sieve; $P = 0.02$) and against medium length particles on the second sieve ($P < 0.01$) of the particle size separator, to a greater extent than cows fed barley. There were no differences in sorting between the barley treatments. Apparent total tract digestibility of DM (64.9 vs. 60.5; $P < 0.001$; Table 2. 5), OM (69.7 vs. 65.7%; $P < 0.001$), starch (97.7 vs. 94.1%; $P < 0.001$), and EE (83.3 vs. 77.6%; $P < 0.001$) was higher for the CM treatment compared to barley grain treatments. Apparent total tract digestibility of starch was greater (95.6 vs. 92.6%; $P < 0.001$) and that of EE (75.7 vs. 79.5%; $P < 0.001$) was lower for Xena compared to Dillon treatment. Apparent total tract digestibility of CP and NDF was not affected by treatment.

Daily yields of milk (40.4 vs. 37.4 kg/d; $P < 0.01$), milk protein (1.20 vs. 1.12 kg/d; $P < 0.001$) and milk lactose (1.85 vs. 1.74 kg/d; $P < 0.01$) were higher for cows fed CM than for cows fed barley grain. Milk fat yield (1.33 vs. 1.24 kg/d; $P = 0.10$) and milk lactose concentration (4.65 vs. 4.55%; $P = 0.06$) tended to be higher for cows fed CM diets compared to cows fed barley grain. Daily yields of milk (38.5 vs. 36.2 kg/d; $P = 0.04$), milk protein (1.18 vs. 1.07 kg/d; $P < 0.001$) and lactose (1.80 vs. 1.69 kg/d; $P < 0.01$) were higher for cows fed Xena compared to those fed Dillon. Concentrations of milk protein (3.08 vs. 2.89%; $P < 0.001$) and milk lactose (4.62 vs. 4.49%; $P = 0.03$) were also higher for Xena compared to Dillon. However, milk fat concentration tended to be higher (3.47 vs. 3.23%; $P = 0.08$) for cows fed Dillon than Xena. The SCC and MUN were not affected by treatment.

Cows fed Dillon gained more BW (0.16 vs. -0.53 kg/d) compared to cows fed Xena although a treatment effect was not observed for the comparison between CM and barley grain treatments. The BCS was also not affected by treatment. Cows fed CM had a higher 4% FCM yield (36.1 vs. 33.6 kg/d; $P < 0.01$) and milk energy output (26.5 vs. 24.2 Mcal/d; $P < 0.001$) compared to cows fed barley. Energy utilization

efficiency, defined as net energy output of milk divided by net energy intake was higher for barley compared to CM treatment (86.9 vs. 80.1%; $P = 0.02$). Plasma concentrations of glucose and NEFA were not affected by treatment (Table 2. 4). Plasma insulin concentration was higher for cows fed Xena than Dillon (8.50 vs. 5.91 μIU/mL; $P < 0.01$) but no difference was observed between CM and barley grain treatments.

2.4. Discussion

2.4.1. Corn vs. Barley

Replacing corn with barley reduced DMI of lactating dairy cows by about 2 kg/d. This reduction in DMI is similar to that reported in some studies (Casper and Schingoethe, 1989; McCarthy et al., 1989; Casper et al., 1990; Khorasani et al., 1994; Yang et al., 1997b), although other studies have not observed low intake of cows fed barley diets (De Peters and Taylor, 1985; Grings et al., 1992; Casper et al., 1999). Inconsistent effects of grain source on DMI can be attributed to the proportion of grain in the diet (DeVisser et al., 1990; Grings et al., 1992), the starch concentration of the diet (Tommervik and Waldern, 1969; Rode and Satter, 1988; DeVisser et al., 1990), and forage particle length (Rode and Satter, 1988). Diets containing higher concentrations of forage NDF promote chewing, salivation, and high rumen pH (Mertens, 1997), which mask some effects caused by differences in fermentability of diets in the rumen.

Milk yield was greater for cows fed CM although efficiency for milk production, defined either as milk yield / DMI or NE_L milk / NE_L intake, was not greater for CM treatment. Thus, higher milk yield of cows fed CM is attributed to greater DMI. Some previous research also reported a higher milk yield for corn based diets compared to barley based diets (McCarthy et al., 1989; Overton et al.,

1995; Casper et al., 1999). However other research observed no treatment effect on milk yield (Grings et al., 1992; Khorasani et al., 1994). The lack of effect of grain source on milk lactose, fat and protein concentrations is consistent with previous studies in which the effects of replacing corn with barley resulted in inconsistent effects on milk components. For example, Casper et al. (1990) reported milk fat increased when corn replaced barley, but other research (De Peters and Taylor, 1985; Bilodeau et al., 1989; Casper and Schingoethe, 1989; McCarthy et al., 1989) found no differences in milk fat content. Milk lactose and protein were not different between barley and corn based diets in previous studies (De Peters and Taylor, 1985; Bilodeau et al., 1989; McCarthy et al., 1989; Grings et al., 1992).

2.4.2. Effects of Barley Grain Treatments

We used two barley cultivar lots, Dillon and Xena, to test our hypothesis that less fermentable barley grain would increase productivity of dairy cows compared with more fermentable barley grain. The Xena barley used in this study had greater starch concentration (58.7 vs. 50.0% of DM) and greater in vitro 6-h starch digestibility (78.0 vs. 73.5 %) compared with Dillon barley. Although actual ruminal starch digestion was not determined in this study, the higher plasma insulin concentration (8.50 vs. 5.91 μIU/ml) and apparent total tract starch digestibility (95.6 vs. 92.6 %) for cows fed Xena compared with Dillon are consistent with the expected greater ruminal starch digestion for Xena compared with Dillon. Furthermore, a companion study conducted with the same lots of Xena and Dillon found that ruminally cannulated dairy cows fed Xena had greater propionate concentration in ruminal fluid and longer time that rumen pH was below 5.8 compared with cows fed Dillon (Silveira et al., 2007).

We expected less reduction in feed intake for cows fed Dillon compared with Xena due to the slower ruminal fermentation of Dillon. Contrary to expectations, DMI was not different between Xena and Dillon. Our findings are in agreement with Yang et al., (1997a) who compared hulless barley with hulled barley and corn grain. They reported that DMI was reduced by 1.3 kg/d for cows fed barley compared with corn grain, but they did not observe a difference in DMI between barley grain treatments although hulless barley was more slowly digested in the rumen than hulled barley (Yang et al., 1997a). Excess fermentation in the rumen is generally considered as the primary cause of lower DMI and milk yield for cows fed barley grains compared with corn grain (McCarthy et al., 1989; Overton et al., 1995). Ruminal concentration of propionate is typically higher for barley based diets than corn based diets (McCarthy et al., 1989; Overton et al., 1995), which is consistent with the principle that propionate has hypophagic effects in ruminant animals (Allen, 2000), and decreases energy intake (Oba and Allen, 2003). However, barley grains that are less fermentable in the rumen did not prevent reduction in DMI in the studies of Yang et al. (1997b) and ours, which indicates that lower DMI often observed for cows fed barley grains cannot be solely attributed to excess fermentation or hypophagic effects of propionate.

Because DMI was not different between Xena and Dillon, the greater milk yield of cows fed Xena compared with Dillon is attributed to higher starch content of the Xena diet coupled with its higher total tract starch digestibility. Cows fed Xena tended to have a lower milk fat concentration, which is consistent with the expected greater starch digestion in the rumen for Xena treatment. As rumen pH declines, the biohydrogenation of unsaturated fatty acids is disturbed, increasing the accumulation of *trans*-C18:1, which depress de novo fatty acid synthesis in the mammary gland (Griinari et al., 1998). Greater milk protein concentration for the Xena treatment can

be attributed to the expected greater ruminal starch digestion. The higher propionate concentration in ruminal fluid from cows fed Xena (Silveira et al., 2007) would have favored the use of propionate by the liver for gluconeogenesis to a greater extent than for cows fed Xena. Increased gluconeogenesis would have a sparing effect on amino acids, increasing availability of amino acids for milk protein synthesis in the mammary gland. Greater milk protein concentration for cows fed Xena compared with those fed Dillon can be also attributed to greater plasma insulin concentration (Mackle et al., 1999).

Effects of barley grain cultivar on animal productivity have been studied for beef cattle. Bradshaw et al. (1996) fed two barley cultivars with the same test weight (67 kg/hL), Steptoe and Klages, to growing steers (31% barley in the diet) and finishing steers (87% barley in the diet) and found no differences in steer performance. However, when six studies were performed over five years, Klages increased weight gain in one study, decreased weight gain in two studies, and no resulted in no difference in gain in the remaining three studies (Krecher et al., 1986). Ovenell-Roy et al. (1998a) and Ovenell-Roy et al. (1998b) fed six barley cultivars to steers in finishing diets and found that digestibility of NDF was the main factor contributing to cultivar differences, where the cultivar with lowest NDF digestibility tended to have lower starch and digestible energy. Covered (Leduc, 61.9 kg/hL) and hull-less (Condor, 76.1 kg/hL) barley was fed to steers in finishing diets (77% barley in the diet) and gain was found to be similar for both cultivars, however cattle fed Leduc had higher intake, leading to a decreased feed efficiency and dietary energy density (Zinn et al., 1996). A study evaluating three cultivars: Gunhilde (two-row, 64.5 kg/hL), Harrington (two-row malting, 63.1 kg/hL) and Medallion (six-row, 61.9 kg/hL) concluded that Medallion had the better feed conversion when fed to steers in

finishing diets (80% barley in the diet) followed by Gunhilde and Harrington (Boss and Bowman, 1996).

Some evaluated effects of barley grain differing in nutrient composition using lactating dairy cows (Yang et al., 1997a, b; Foley et al., 2006), but most research evaluating barley grain using dairy cows compared the feeding value of barley grain with that of corn grain, and the variations within barley grain have been overlooked. We found significant differences in milk protein concentration and plasma insulin concentration between barley grain treatments, but not between CM and barley treatments. These observations indicate that selection of barley grain can affect some nutrient metabolism and milk production responses to a greater extent than selection of grain type (i.e., corn vs. barley). Selection of barley cultivar can be a viable management option to enhance productivity of dairy cows, but our research data need to be interpreted with caution. Although we found greater milk yield for cows fed Xena compared with those fed Dillon, it cannot be concluded that Xena is a better cultivar than Dillon for lactating dairy cows. Effects of growing environment and interactions of barley genetics with its growing environment are expected to greatly affect physical and chemical characteristics of barley grains. In the current study, Xena was compared with Dillon, but it is not known whether the lots of grain used in this study are representative of each cultivar. However, our study clearly demonstrates that selection of barley grain can affect productivity of lactating dairy cows. Further research is warranted to identify the quality traits of barley grain that influence productivity of lactating dairy cows.

2.5. Conclusion

Cows fed corn had higher DMI and milk production compared with those fed barley grain. However, despite expected lower ruminal starch digestion due to lower

starch content and reduced fermentability, cows fed Dillon had similar DMI as cows fed Xena. Milk yield and milk protein concentration were lower for cows fed Dillon compared with cows fed Xena, indicating that reducing ruminal starch digestion of barley grain may not improve productivity of lactating dairy cows. Barley grain fed to ruminants needs to be carefully selected due to large variations in nutrient composition across the lots and their effects on productivity of lactating dairy cows. Future research needs to identify the quality traits of barley grain that affect productivity of animals.

Table 2. 1. Nutrient composition of the barley grains used to formulate experimental diets

Item	CM[1]	Dillon	Xena
Test Weight, kg/hL	-	61.5	75.3
DM, %	95.1	92.4	91.6
CP, % DM	12.8	10.3	12.6
Soluble Protein, % CP	2.2	1.6	2.5
ADF, % DM	3.7	9.2	7.8
NDF, % DM	14.3	19.0	27.0
Ether extract, % DM	2.8	2.2	2.0
Ash, % DM	1.6	3.0	2.6
Starch, % DM	60.4	50.0	58.7
In vitro 6-h starch digestibility, % starch	71.1	73.5	78.0

Table 2. 2. Ingredients and nutrient composition of experimental diets containing either corn mixture (CM) [1], steam-rolled Dillon, or steam rolled Xena

		Barley	
Item	CM	Dillon	Xena
Ingredients			
Barley silage	26.6	27.3	27.4
Alfalfa hay	15.8	16.1	16.2
Basal concentrate mix[2]	17	17.5	17.5
CM	40.6	...	...
Dillon, steam-rolled	...	39.1	...
Xena, steam-rolled	...	...	38.9
Nutrient composition			
Forage NDF	25.1	25.3	25.5
NDF	33.0	39.3	37.5
CP	19.4	19.4	19.6
Starch	28.7	22.3	25.3
EE	3.0	2.6	2.3

[1] Corn mixture contained 87.5% dry ground corn, 11.4% beet pulp, and 1.1% urea (DM basis),

[2] Basal concentrate mix contained 10% beet pulp, 10% Megalac®, 0.5% vegetable oil, 17.5% fish meal, 14% corn meal, 22% canola meal, 3.8% limestone, 1.2% magnesium oxide, 1.9% sodium bicarbonate, 7.7% calcium phosphate, and 11.4% premix of microminerals and vitamins (DM basis)

Table 2. 3. The DMI and performance of lactating dairy cows fed diets containing either corn mixture (CM) [1], steam-rolled Dillon, or steam rolled Xena

Item	CM	Barley		SE	*P* value	
		Dillon	Xena		CM vs. Barley	Dillon vs. Xena
DMI (kg/d)	23.6	21.4	21.8	0.69	< 0.001	0.35
Sorting index[2]						
1st (top) sieve (19-mm)	1.03	0.96	0.93	0.027	0.02	0.46
2nd sieve (8-mm)	0.96	1.02	1.03	0.016	< 0.01	0.51
3rd sieve (1.18 mm)	1.02	1.02	1.01	0.009	0.65	0.45
Collection pan	1.15	1.05	1.03	0.056	0.13	0.75
Yield (kg/d)						
Milk	40.4	36.2	38.5	1.22	< 0.01	0.04
Milk fat	1.33	1.26	1.23	0.059	0.10	0.55
Milk protein	1.20	1.07	1.18	0.039	< 0.001	< 0.001
Milk lactose	1.85	1.69	1.8	0.067	< 0.01	< 0.01
Milk composition (%)						
Milk fat	3.39	3.47	3.23	0.133	0.72	0.08
Milk protein	2.99	2.89	3.08	0.060	0.85	< 0.001
Milk lactose	4.65	4.49	4.62	0.055	0.06	0.03
SCC (10^3/mL)	147	331	229	113.7	0.21	0.78
MUN (mg/dL)	14.7	14.7	14.3	0.35	0.66	0.28
4% FCM yield (kg/d)	36.1	33.3	33.9	1.10	< 0.01	0.54
NE_L milk output (Mcal/d)[3]	26.5	23.6	24.8	0.80	< 0.001	0.12
Milk yield / DMI	1.74	1.71	1.77	0.052	0.90	0.24
NE_L milk output / NE_L intake[4]	0.80	0.86	0.88	0.263	0.02	0.53
BW change (kg/d)	-0.23	0.12	-0.53	0.121	0.87	< 0.001
BCS change (/21d)	0.02	0.01	0.06	0.029	0.63	0.24

[1] Corn mixture contained 87.5 % dry ground corn, 11.4% beet pulp, and 1.1% urea (DM basis)

[2] Sorting index = actual intake / expected intake (< 1 sorting against, > 1 sorting for and = 1 no sorting)

[3] Milk energy output was calculated from measured milk yield and concentrations of milk fat, protein, and lactose according to NRC (2001): NE_L (Mcal/kg)= 0.0929 × Fat % + 0.0547 × Crude Protein % + 0.0395 × Lactose %

[4] NE_L intake was calculated from DMI and measured NE_L content of diets; Digestible energy content of diets was first calculated from nutrient components actually digested in the total tract, and then converted to NE_L according to the NRC (2001): ME_P (Mcal/kg) = 1.01 x DE (Mcal/kg) – 0.45; NE_L (Mcal/kg) = [0.703 x ME_P (Mcal/kg)] – 0.19

Table 2. 4. Plasma metabolite concentrations of lactating dairy cows fed diets containing either corn mixture (CM) [1], steam-rolled Dillon, or steam rolled Xena

		Barley			*P* value	
Plasma metabolite	CM	Dillon	Xena	SE	CM vs. Barley	Dillon vs. Xena
NEFA (meq/L)	102.0	120.7	104.4	0.786	0.32	0.24
Glucose (mg/dL)	61.3	60.3	61.1	0.660	0.32	0.24
Insulin (mIU/mL)	7.44	5.91	8.50	0.637	0.64	< 0.01

[1] Corn mixture contained 87.5 % dry ground corn, 11.4% beet pulp, and 1.1% urea (DM basis)

Table 2. 5. Apparent total tract nutrient digestibility of lactating dairy cows fed diets containing either corn mixture (CM) [1], steam-rolled Dillon, or steam rolled Xena

		Barley			*P* value	
Digestibility (%)	CM	Dillon	Xena	SE	CM vs. Barley	Dillon vs. Xena
DM	64.9	60.1	60.8	0.83	< 0.001	0.52
OM	69.7	65.5	66.0	0.72	< 0.001	0.61
Starch	97.7	92.6	95.6	0.36	< 0.001	< 0.001
EE	83.3	79.5	75.7	0.66	< 0.001	< 0.001
CP	68.2	67.8	66.7	0.74	0.32	0.34
NDF	46.2	46.9	46.5	1.30	0.73	0.80

[1] Corn mixture contained 87.5 % dry ground corn, 11.4% beet pulp, and 1.1% urea (DM basis)

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3.0 SELECTION OF BARLEY GRAIN AFFECTS RUMINAL FERMENTATION, STARCH DIGESTIBILITY AND PRODUCTIVITY OF LACTATING DAIRY COWS

3.1. Introduction

Barley grain varies in chemical composition; it ranges from 7.5 to 15% CP (LaFrance and Watts, 1986), from 17.3 to 32.1% NDF (Ovenell-Roy et al., 1998), from 45.9 to 62.8% starch (Ovenell-Roy et al., 1998), and from 20.0 to 64.4 %/h for the rate of in situ DM degradation (Khorasani et al., 2000). Yang et al. (1997a, b) compared hulled and hull-less barley grain, and reported that milk production was not affected by treatment despite the distinctive differences in chemical and physical characteristics of the barley grains. However, in a study that compared two lots of barley cultivars, Xena and Dillon, cows fed Xena had higher total tract starch digestibility, milk yield, milk protein and lactose concentrations and a tendency to have lower milk fat compared with cows fed Dillon (Silveira et al., 2007). Although that study demonstrated that selection of barley grain affects productivity of lactating dairy cows, the treatment effects on ruminal pH and fermentation, nutrient digestibility in the rumen, and the efficiency of microbial protein production were not evaluated. We hypothesized that lower milk fat content for cows fed Xena resulted from greater starch digestion in the rumen and lower ruminal pH. We also hypothesized that selection of barley grain can affect rumen fermentation and productivity to a similar extent as altering the proportion of grain in the diet, the most common practical approach to changing ruminal fermentation and milk production. The objective of our study was to understand the mechanism by which Xena treatments increased milk production by evaluating rumen fermentation, ruminal and total tract digestion, and microbial protein production of lactating dairy cows. Two dietary

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concentrations of barley grains were used so that these effects were evaluated at high and low dietary starch concentrations.

3.2. Material and Methods

3.2.1. Cows, Diets and Treatments

The study was conducted at the Agriculture and Agri-Food Canada Research Centre in Lethbridge, AB, and cows were cared for according to the guidelines of the Canadian Council on Animal Care. Four multiparous Holstein cows in early lactation (46 ± 14 DIM, $\overline{X}$ ± SD) that were surgically fitted with ruminal and duodenal cannulas and four primiparous Holstein cows in early to mid lactation (123 ± 69 d DIM) that were fitted with ruminal cannulas were used. The ruminal cannulas measured 10 cm in diameter and were constructed of soft plastic (Bar Diamond, Parma, ID). Duodenal cannulas were T-shaped and were placed proximal to the common bile and pancreatic duct, approximately 10 cm distal to the pylorus. The multiparous and primiparous cows were separately assigned to one of two 4 × 4 Latin squares balanced for carryover effects. Both squares were conducted simultaneously. Treatments were two levels of dietary starch concentration (30 vs. 23% dietary DM) and two steam-rolled barley grains (Dillon vs. Xena). At the beginning of the experiment, BW of the multiparous cows averaged 628 ± 35 kg and BCS was 3.0 ± 0.40, and BW of the primiparous cows averaged 628 ± 55 kg and BCS was 2.8 ± 0.30. Treatment periods were 16 d with the final 6 d used to collect samples and data.

Two lots of barley grain cultivars, Xena and Dillon, were obtained from a local grain company. These lots were selected because of their distinctive differences in physical and chemical characteristics (Table 3. 1), and were the same lot utilized for the companion study (Silveira et al., 2007). All experimental diets were formulated to contain

18.3% CP and 20.0% forage NDF (Table 3. 2), and to meet or exceed the other nutrient requirements of the cows (NRC, 2001). Diets were fed as a TMR. Throughout the experiment, cows were housed in tie stalls with continuous access to water, fed three times daily (0600, 1200 and 1800 h) for ad libitum intake, and milked in their stalls twice daily (0600 and 1700 h). Animals were allowed to exercise once daily (0900 h) for 2 h.

3.2.2. Data and Sample Collection

Body weight was recorded immediately prior to the start of the first period and on the last day of each period. Body condition score was determined by a trained investigator one day prior to the start of each period and on the last day of each period, using a five-point scale (1=thin to 5= fat; Wildman et al., 1982). The amount of feed offered and orts were weighed and recorded daily. Representative samples of all dietary ingredients, TMR and orts (approximately 12.5% of feed refused, as-fed basis) were sampled daily during the collection period. The orts samples were pooled and one sample was retained per cow per period. The DM content of barley silage and alfalfa hay was determined weekly to adjust allocation of forages to maintain consistent forage to concentrate ratio on a DM basis. Milk yield was measured daily and averaged over the collection period. Milk was sampled at every milking on d 13, 14, 15 and 16 of each period.

Ruminal pH was measured continuously for three days (d 14 to 16) using the Lethbridge Research Centre ruminal pH measurement system (Penner et al., 2006). Ruminal pH readings were taken every 30 s and averaged every minute. Ruminal digesta samples (250 ml per site) were obtained from four locations within the rumen (reticulum, dorsal and ventral sac, and the fiber mat), composited, and squeezed through a nylon mesh (1 mm pore size) at 0600, 1800 and 2400 h on d 13, at 1400 h on d 14, and at 0900 and 2100 h on d 15. Five milliliters of filtrate was preserved by adding 1 ml

of 25% (wt/vol) HPO_3 to determine VFA concentrations and 5 ml of filtrate was preserved by adding 1 ml of 1% (wt/vol) H_2SO_4 to determine ammonia concentration. The samples were stored at -20°C until the analyses.

Duodenal digesta flow and fecal flow of nutrients were estimated using $YbCl_3$ (GFS Chemicals, Inc., Powell, OH) as an external marker. Ammonium sulphate labelled with ^{15}N ($[^{15}NH_4]_2SO_4$, 10.6 atom % ^{15}N; Isotec-Sigma-Aldrich Family, St. Louis, MO) was used as a ruminal microbial marker. During d 7-16 the marker solution containing Yb and ^{15}N were continuously infused into the rumen of cows via the ruminal cannula using an automatic pump. Daily amounts infused were 1.5 g of Yb and 180 mg of ^{15}N dissolved in 550 ml of water for each animal. Ruminal (800 ml from the dorsal, middle and ventral parts of the rumen), duodenal (300 ml), and fecal samples (100 g) were collected at 0600, 1200, 1800, 2400 h on d 13, 0800, 1400, 2000 h on d 14, 0200, 0900, 1600, 2100 h on d 15 and at 0400 h on d 16. Twelve subsamples were pooled per cow per period, providing representative ruminal, duodenal, and fecal samples that accounted for diurnal variation. Ruminal samples were immediately squeezed through nylon mesh (1 mm pore size). Ruminal particles were then blended (400 g of ruminal particles plus 400 ml of 0.9% NaCl) in a Waring blender (Waring Products Division, New Hartford, CT) for 1 min and then squeezed through a nylon mesh (1 mm pore size). Filtrates obtained by squeezing the ruminal samples and the filtrate obtained from squeezing the blended homogenate were combined and centrifuged (800 x *g* for 15 min at 4°C) to remove protozoa and remaining fine feed particles, and the supernatant was centrifuged (27,000 x *g* for 30 min at 4°C) to obtain a mixed ruminal bacteria pellet. Microbial pellets were pooled by period for each cow, freeze-dried and ground using a ball mill (Mixer Mill MM2000; Retsch, Haan, Germany) to determine the ratio of ^{15}N to N, starch, and OM. Duodenal samples were pooled by cow within each period, mixed using a blender (model MX-9100, Toshiba, Tokyo, Japan) and freeze-dried. Fecal samples were

collected from the rectum of each cow, dried in a forced air oven at 55°C, and pooled by cow within each period. Dried duodenal and fecal samples were ground through a 1-mm screen (Thomas-Wiley, Philadelphia, PA).

3.2.3. Sample Analysis

Diet ingredients, orts, duodenal digesta, and fecal samples were analyzed for concentrations of DM, ash, NDF, ether extract (**EE**), CP and starch. The DM concentration was determined by drying sample at 135°C for 2 h. Ash concentration was determined after 5 h at 500°C in a furnace. The NDF concentrations were determined by the Van Soest method with amylase and sodium sulfite (Van Soest et al., 1991). Crude protein was quantified by flash combustion with gas chromatography and thermal conductivity detection (Carlo Erba Instruments, Milan, Italy; Handbook of Food Analytical Chemistry, 2005), ^{15}N enrichment in rumen bacterial pellets and duodenal samples were also determined by flash combustion (Carlo Erba Instruments, Milan, Italy) with isotope ratio mass spectrometry (VG Isotech, Middlewich, England). Starch was measured by an enzymatic method (Karkalas, 1985) after samples were gelatinized with sodium hydroxide; glucose concentration was measured using a glucose oxidase/peroxidase enzyme (Sigma Cal. No. P7119), and dihydrochloride (Sigma Cal. No. F5803). Absorbance was determined with a microplate reader (SpectraMax 190, Molecular Devices Corp., Sunnyvale, CA). The EE was determined using a Goldfisch extraction apparatus (Labconco, Kansas City, MO, USA; Handbook of Food Analytical Chemistry, 2005). Ytterbium concentration in duodenal and fecal samples was determined using inductively coupled plasma optical emission spectroscopy according to the (AOAC, 1990) with modification such that no KCl was used during sample digestion.

Milk fat, protein, lactose and SCC concentrations were measured with infrared spectroscopy by Edmonton-Alberta DHIA (Milk O Scan 605), and milk urea nitrogen (mg/dL) was determined with an automated IR Fossomatic 400 Milk Analyzer (Foss

North America, Brampton, Ontario). Ruminal and duodenal ammonia concentrations were determined by the method described by Fawcett and Scott (1960). Ruminal VFA concentrations were determined by gas chromatography (Varian 3700; Varian Specialities, Ltd., Brockville, ON) using a 15-m fused silica column (DB-FFAP column; J and W Scientific, Folsom, CA).

The TMR and orts were analyzed for particle size distribution using the Penn State Particle Separator (NASCO, Fort Atkinson, WI) to determine extent of sorting, which was expressed as a sorting index. The sorting index was calculated as the actual intake / expected intake for each portion retained on the individual sieves. Expected intake was calculated as the particle size distribution of the TMR (%) × actual as-fed intake. Actual intake was calculated as the amount of feed offered × particle size distribution in the TMR (%) – the amount feed refused × the particle size distribution in the orts samples (%). A sorting index of 1 indicates no sorting, a sorting index of less than one indicates sorting against, and greater than 1 indicates sorting for, particles on the particular screen.

3.2.4. Statistical Analysis

All data were analyzed using the fit model procedure of JMP (version 5.1, SAS Inc., Cary, NC) according to the following model:

$$Y_{ijkl} = \mu + S_i + C(S)_{ij} + P_k + T_l + e_{ijklm}$$

where μ= overall mean

S_i= fixed effect of square (i=1 to 2)

$C(S)_{i(j)}$= random effect of cow nested in squares (j=1 to 8)

P_k= fixed effect of period (k=1 to 4)

T_l= fixed effect of treatment (l= 1 to 4)

e_{ijklm}= residual, assumed to be normally distributed.

Square × treatment interaction had been originally included in the model, but it was removed because the interaction was not significant for all response variables. Outliers were removed according to the Jackknife distance analysis and orthogonal contrasts were made to evaluate the effect of dietary starch concentration, barley grain and their interactions. Treatment effect and its tendency were considered significant at $P < 0.05$ and $P < 0.10$, respectively

3.3. Results

The DMI was affected neither by dietary starch concentration nor barley grain treatment, and averaged 20.0 kg/d (Table 3. 3). Sorting index, BW and BCS change were not affected by treatment either. Milk yield was not affected by treatment, and averaged 31.0 kg/d. Milk fat concentration (3.55 vs. 3.29%) was greater for cows fed Dillon compared with Xena, but it was not affected by dietary starch concentration. Consequently, 4% FCM tended to be higher for cows fed Dillon compared with Xena (28.7 vs. 27.7 kg/d). The concentrations of milk protein and lactose, SCC and MUN were not affected by treatment.

High starch diets tended to decrease mean ruminal pH (6.10 vs. 6.17; Table 3. 4), and increased the duration that pH was below 5.8 (6.4 vs. 4.2 h/d) and the area below pH 5.8 (5715 vs. 3375 pH × s) compared with low starch diets. However, mean ruminal pH was not affected by barley grain treatment, although cows fed Xena had longer duration that ruminal pH was below 5.8 (6.6 vs. 4.0 h/d) and that ruminal pH was between 5.5 and 5.2 (1.97 vs. 1.18 h/d) and tended to have lower minimum pH (5.45 vs. 5.59) and greater standard deviation for daily pH measurements (0.32 vs. 0.28) compared with cows fed Dillon.

Ruminal acetate molar proportion was lower (60.1 vs. 62.0 and 60.2 vs. 61.8 mol/100mol; Table 3. 4), and propionate molar proportion was greater (24.4 vs. 22.1 and

24.3 vs. 22.2 mol/100 mol) for cows fed Xena or high starch diets compared with cows fed Dillon or low starch diets, respectively. Total VFA molar concentration was not affected by treatment.

Apparent total tract digestibilities of DM, OM, EE and CP were not affected by treatment (Table 3. 5). Total tract starch digestibility was higher for cows fed high starch (94.3 vs. 93.0%) or Xena (94.3 vs. 93.0%) diets compared to low starch or Dillon diets, respectively. Dillon treatments had greater total tract NDF digestibility compared with Xena treatments (57.9 vs. 52.3%).

Organic matter truly digested in the rumen, and OM flow to the duodenum and apparently digested in the intestines, were not affected by treatment (Table 3. 7). Cows fed Xena tended to increase the amount of starch truly digested in the rumen (3.85 vs. 3.19 kg/d) compared to cows fed Dillon diets. Cows fed low starch diets shifted site of starch digestion from the rumen to the intestines; true starch digestibility in the rumen was lower (49.2 vs. 70.2%), but intestinal starch digestibility was greater (42.2 vs. 23.2 %) compared with high starch diets.

Cows fed Dillon (6.92 vs. 6.32 kg/d) or low starch diets (6.90 vs. 6.34 kg/d) had higher NDF intake compared to cows fed Xena or high starch diets, respectively. Cows fed low starch diets also had a tendency to have higher NDF digested in the rumen compared to high starch diets (1.99 vs. 1.41 kg/d).

True ruminally digested organic matter **(TRDOM)**, N intake, duodenal passage of ammonia N and non-ammonia N **(NAN)**, microbial N produced daily, microbial efficiency (g of microbial N / kg of TRDOM) and N digested in the total tract were not affected by treatment (Table 3. 9). Cows fed high starch (11.5 vs. 8.7 mg/dL) or Dillon (11.3 vs. 8.9 mg/dL) diets had higher rumen ammonia concentrations compared to cows fed low starch or Xena diets. Duodenal passage of non-ammonia-non-microbial N (**NANMN**) was higher for cows fed Xena diets compared to cows fed Dillon when expressed as a

quantity (176 vs. 100 g/d), as a percentage of N intake (26.1 vs. 15.9%), and as a percentage of duodenal NAN flow (27.9 vs. 19.0%).

3.4. Discussion

Milk yield was not affected by barley starch availability in the rumen or the total digestive tract. This finding is contrary to our previous observation that cows fed Xena had higher yield of milk containing higher concentrations of protein and lactose (Silveira et al. 2007). The primary purpose of the current study was to evaluate the digestion parameters associated with the milk response of cows fed Xena, a barley grain cultivar with high expected ruminal degradability. Failure to observe a consistent milk yield effect between these two studies might be partly explained by the use of cannulated cows in the present study. Milk yield of intestinally cannulated animals is generally lower than for intact cows. The average milk yield was 31.0 kg/d for cows used in the current study, which is lower than in our previous study (38.4 kg/d). Thus, milk production in the present study may not have been limited by energy intake or metabolizable protein supplied from diets; rather the limitation may have been the milk potential of the cows.

We hypothesized that cows fed Xena would have lower ruminal pH compared to cows fed Dillon, and that selection of barley grain can affect ruminal fermentation and productivity of cows to a similar extent as altering dietary starch concentration. The duration of ruminal pH below 5.8, ruminal VFA profile, and total tract starch digestibility were affected by both dietary starch concentration and barley grain treatment. In addition, it is noteworthy that milk fat yield and concentration were affected by barley grain treatment, but not by dietary starch concentration. These results demonstrate that selection of barley grain can affect ruminal fermentation and milk production to an extent at least as great as dietary starch concentration.

3.4.1. Rumen Fermentation and Milk Fat Depression

There are two main theories explaining milk fat depression. The first one is the glucogenic-insulin theory: when high concentrate diets are fed to lactating dairy cows propionate production in the rumen increases, which stimulates hepatic glucose synthesis (Bergman et al., 1966; Brockman, 1985; Brockman and Laarveld, 1990) and insulin release (Bergman et al., 1970). Enhanced plasma insulin concentration increases nutrient uptake by adipose tissues (Jenny et al., 1974; Sutton et al., 1988; Van Soest, 1994), and less substrates are available to the mammary gland for de novo fatty acid synthesis, decreasing milk fat production. The second theory explaining milk fat depression is referred as the trans-fatty acid theory: when high grain diets are fed, ruminal pH drops and incomplete biohydrogenation of unsaturated fatty acids allows accumulation of trans-$C_{18:1}$ in the rumen (Griinari et al., 1998). These trans fatty acids cause a reduction of de novo fatty acid synthesis in the mammary gland, decreasing milk fat production (Gaynor et al., 1995; Griinari et al., 1998).

The milk fat depression observed for cows fed Xena can be attributed to both theories. Although fatty acid composition was not determined in this study, Xena treatments increased the duration that ruminal pH was below 5.8 and tended to decrease the daily minimum pH, which could have caused an accumulation of trans-$C_{18:1}$, and led to milk fat depression. However, milk fat was not depressed for cows fed high starch diets although they increased the duration that ruminal pH was below 5.8, thus low rumen pH itself may not explain the milk fat depression observed in our study. It is noteworthy that the fluctuation in rumen pH, measured as the standard deviation of ruminal pH measurements, tended to be greater for cows fed Xena compared to cows fed Dillon ($P < 0.06$), but was not affected by dietary starch concentration. Greater fluctuation in ruminal pH may indicate a more pulsatile nutrient supply from the rumen, because ruminal pH affects the rate of VFA absorption. This pattern is consistent with

the observation that cows fed Xena had greater plasma insulin concentration compared to cows fed Dillon (Silveira et al., 2007). Milk fat depression observed for cows fed Xena, but not for cows fed high starch diets, can be at least partly attributed to the glucogenic theory.

Daily mean ruminal pH was not affected by treatment although the amount of starch truly digested in the rumen varied from 2.25 to 4.94 kg/d among treatments. All experimental diets were formulated to contain the same concentration of forage NDF, which stimulates chewing and rumination, and increases saliva secretion (NRC, 2001). Thus, all treatments were provided with the same buffering capacity. It is interesting that daily mean ruminal pH averaged 6.1, which is the pKa of bicarbonate, the primary salivary buffer. These observations indicate that salivary buffer played a dominant role in maintaining similar daily mean ruminal pH across treatments. However, milk fat depression observed for cows fed Xena, despite no differences in daily mean ruminal pH, indicates that mean rumen pH does not necessarily reflect effects of ruminal fermentation on physiological responses including milk fat depression. This finding emphasizes the importance of continuous measurement of ruminal pH. Although barley grain treatment did not affect mean ruminal pH, it affected milk fat production and the duration that rumen pH was below 5.8, which can be determined only by continuous measurement of ruminal pH.

3.4.2. Digestibility and N Metabolism

3.4.2.1. Digestibility

We found that cows fed low starch diets, in which barley grain was replaced by beet pulp, decreased starch digestibility in the rumen. In agreement with our observation, Ipharraguerre et al. (2002) and Voelker and Allen (2003) found that starch digestibility in the rumen decreased when fibrous by-products, soy-hulls and beet pulp

respectively, was added to diets of lactating dairy cows. Voelker and Allen (2003) attributed the linear reduction in true ruminal starch digestibility caused by the increased substitution of high moisture corn with beet pulp to the decreased amylotic enzyme activity in the rumen and to increased starch passage rate caused by greater ruminal fill. Although true ruminal starch digestibility was 20 percentage units greater for cows fed high starch diets compared to low starch diets, the difference in apparent total tract starch digestibility was far less, indicating that a compensatory post ruminal starch digestion occurred for cows fed low starch diets. Previous research has also showed that, unless enzymatic activities limit intestinal starch digestion, compensatory starch digestion in the intestines occurs when less ruminally fermentable grains are fed (Callison et al., 2001; Knowlton et al., 1998; Ying and Allen, 1998).

Starch digestibility in the rumen was not affected by barley grain although Xena had a greater 6-h in vitro starch digestibility than Dillon (78.0 vs. 73.5% starch). Ruminal starch digestibility is also affected by passage rate (Nocek and Tamminga, 1991). Thus, lack of significant effects of barley grain treatment on ruminal starch digestibility may be attributed to faster passage rate of starch for cows fed Xena. Alternatively, the number of animals used to determine starch digestibility in the rumen (i.e., four) might not have been sufficient to detect treatment effects with statistical significance. However the amount of starch digested in the rumen was greater for Xena treatments, which is consistent with the lower minimum pH and the longer duration of pH below 5.8.

Apparent total tract NDF digestibility was lower for cows fed Xena, which could be explained by the lower ruminal pH and higher starch concentrations of Xena treatments since low ruminal pH and presence of starch negatively affect NDF digestion (Grant and Mertens, 1992). However ruminal NDF digestion was not affected by barley grain treatment, thus the reasons for decreased total tract NDF digestibility for Xena treatments are not known.

3.4.2.2. N metabolism

In the companion study, milk protein yield was greater for cows fed Dillon (Silveira et al., 2007), therefore we expected that barley grain treatment would affect N metabolism. However, MN flow to the duodenum and microbial efficiency were not affected by treatment in the current study. Cows fed Xena or high starch diets had longer duration that ruminal pH was below 5.8. Lower pH can cause energy spilling by using additional energy to maintain intracellular pH and decreasing the energy available for microbial growth (Strobel and Russell, 1986). But, barley grain treatment did not affect microbial efficiency in the current study.

Greater ruminal ammonia concentration for high starch diets was not expected. These higher ruminal ammonia concentrations were probably due to greater protein degradability for barley grain compared with beet pulp; barley grain protein has a greater soluble fraction, as well as a faster rate of degradation for the potentially degradable fraction, compared with beet pulp protein (NRC, 2001). Similarly, the greater ruminal ammonia concentration and the decreased duodenal flow of NANMN for Dillon treatments may have resulted from the urea supplementation of the Dillon barley that was used to make the experimental diets iso-nitrogenous .

3.5. Conclusion

The duration that ruminal pH was below 5.8 was longer, ruminal propionate concentration was greater, and ruminal acetate concentration was lower for cows fed Xena or high starch diets compared with cows fed Dillon or low starch diets, respectively. Milk fat yield and concentration were decreased for cows fed Xena, but not affected by dietary starch concentration. The two lots of barley cultivars evaluated in this study affected ruminal fermentation, digestibility, and milk production to an extent as

great as the changes in dietary starch concentration. These observations demonstrate that selection of barley grain lots for quality is an important management tool to optimize milk production.

Table 3. 1. Nutrient composition of barley grains used to formulate experimental diets.

Item	Dillon	Xena
DM, %	92.4	91.6
CP, % DM	10.3	12.6
Available protein, %DM	9.7	12.2
Unavailable protein, %DM	0.5	0.4
NDCP[1], %DM	1.5	1.6
Soluble protein, %CP	1.6	2.5
ADF, %DM	9.2	7.8
NDF, %DM	19.0	27.0
Ether extract, %DM	2.2	2.0
Ash, %DM	3.0	2.6
Lignin, %DM	2.4	2.1
Starch, %DM	50.0	58.7
NFC, %DM	55.4	60.4
Test weight, kg/hL	61.5	75.3
In vitro 6 h starch dig, % starch	73.5	78.0

[1]NDCP : neutral detergent crude protein

Table 3. 2. Ingredient and nutrition composition of experimental diets (% of dietary DM)

	High Starch		Low Starch	
Item	Dillon	Xena	Dillon	Xena
Ingredients				
Barley silage	31.9	31.9	31.9	31.9
Alfalfa hay	8.7	8.7	8.7	8.7
Concentrate mix[1]	18.4	17.9	18.1	17.8
Barley grain	40.8	41.5	27.5	27.9
Urea	0.2	...	0.04	...
Beet pulp	...	...	13.8	13.7
Nutrients				
Forage NDF	20.0	20.0	20.0	20.0
NDF	33.5	30.8	35.2	33.2
CP	18.6	18.1	18.2	18.4
Starch	28.6	32.0	21.7	24.0
Ether extract	2.0	1.8	1.9	1.8
NE_L, Mcal/kg[2]	1.68	1.74	1.64	1.67
Particle size separation				
1st (top) sieve, 19-mm	22.7	20.9	18.3	20.3
2nd sieve, 8-mm	23.8	30.0	30.4	28.3
3rd sieve, 1.18-mm	47.9	44.3	46.1	44.8
Collection pan	5.5	4.9	5.3	6.6

[1] Concentrate mix contained 1.3% canola oil, 26.4% heat-processed canola meal (Alberta Gold, Canbra Foods, Lethbridge, AB), 25.3% heat and xylose treated soybean meal (Soy Pass, LignoTech, Rothschild, WI), 3.5% premix of vitamins and minerals, 3.7% calcium diphosphate, 2.3% sodium bicarbonate, 2.3% limestone, 23.9% corn gluten meal and 11.3% Megalac ® (Arm & Hammer Animal Nutrition Group, Church & Dwight, Princeton, NJ).
[2]Estimated from diet formulation (CPM-Dairy)

Table 3. 3. The effects of dietary starch concentration and barley grain on performance of lactating dairy cows

Item	High Starch		Low Starch		SE	*P* value		
	Dillon	Xena	Dillon	Xena		Starch[1]	BG[2]	INT[3]
DMI (kg/d)	20.1	20.1	19.9	20.2	0.57	0.71	0.49	0.41
Sorting index[4]								
1st (top) sieve, 19-mm	0.90	0.74	0.83	0.87	0.056	0.55	0.19	0.04
2nd sieve, 8-mm	0.99	1.27	1.07	1.05	0.112	0.55	0.27	0.20
3rd sieve, 1.18-emm	0.99	1.05	1.02	1.03	0.018	0.98	0.10	0.24
Collection pan	0.93	0.86	0.84	0.85	0.075	0.50	0.68	0.55
Yield, kg/d								
Milk	30.5	30.6	31.1	31.8	1.62	0.14	0.57	0.59
Milk fat	1.09	0.97	1.08	1.06	0.060	0.23	0.02	0.12
Milk protein	0.97	0.97	0.99	1.01	0.044	0.09	0.78	0.61
Milk lactose	1.41	1.41	1.44	1.47	0.080	0.13	0.56	0.56
4% FCM	28.7	26.7	28.7	28.6	1.40	0.12	0.09	0.12
Milk composition, %								
Milk fat	3.59	3.22	3.51	3.35	0.172	0.78	0.01	0.31
Milk protein	3.2	3.21	3.21	3.18	0.083	0.87	0.79	0.49
Milk lactose	4.62	4.62	4.63	4.63	0.075	0.57	0.94	0.94
SCC, 10^3/ml	346	322	351	238	183.0	0.70	0.52	0.67
MUN, mg/dl	13.3	12.6	12.5	12.2	0.70	0.08	0.15	0.47
BW change, kg/d	0.74	0.48	-0.37	0.68	3.441	0.14	0.22	0.02
BCS change, /16d	0.06	0.03	0	0.09	0.086	1.00	0.72	0.48

[1]Starch: effect of dietary starch concentration
[2]Grain: effect of barley grain, Dillon vs. Xena
[3]INT: interaction of dietary starch concentration and barley grain
[4]Sorting index = actual intake / expected intake (< 1 sorting against, > 1 sorting for and = 1 no sorting)

Table 3. 4. The effects of dietary starch concentration and barley grain on ruminal pH and ruminal fermentation

Item	High Starch		Low Starch		SE	*P* value		
	Dillon	Xena	Dillon	Xena		Starch[1]	BG[2]	INT[3]
Ruminal pH								
Daily minimum pH[4]	5.56	5.38	5.63	5.52	0.122	0.18	0.06	0.65
Daily mean pH	6.15	6.05	6.19	6.15	0.087	0.09	0.13	0.41
Daily maximum pH[4]	6.73	6.71	6.74	6.78	0.073	0.28	0.85	0.40
Daily SD	0.29	0.33	0.28	0.32	0.026	0.58	0.06	0.90
pH<5.8, h/d	4.69	8.16	3.40	5.00	1.551	0.05	0.03	0.40
pH<5.5, h/d	1.78	2.78	1.10	1.68	0.783	0.08	0.12	0.66
pH<5.2, h/d	0.45	0.44	0.06	0.10	0.215	0.07	0.95	0.90
pH between 5.8-5.5, h/d	2.91	5.39	2.29	3.34	0.998	0.16	0.08	0.44
pH between 5.5-5.2, h/d	1.33	2.34	1.04	1.61	0.613	0.17	0.04	0.54
Area <5.8, pH x s/d	4779	6651	2826	3923	1850.2	0.05	0.20	0.73
Area <5.5, pH x s/d	1419	1702	560	881	632.5	0.10	0.54	0.97
Area <5.2, pH x s/d	266	125	1	43	136.5	0.22	0.72	0.51
VFA								
Total VFA, mM	132	130	132	134	3.563	0.54	0.80	0.42
VFA molar proportions, mol/100 mol								
Acetate	61.4	59.0	62.5	61.1	1.387	< 0.01	< 0.01	0.37
Propionate	22.7	25.8	21.4	22.9	1.782	< 0.01	< 0.01	0.26
Isobutyrate	0.90	0.83	0.80	0.78	0.019	< 0.001	0.02	0.24
Butyrate	11.6	10.8	11.9	11.9	0.629	0.14	0.34	0.44
Isovalerate	1.52	1.45	1.48	1.30	0.077	0.19	0.11	0.48
Valerate	1.45	1.72	1.41	1.59	0.0946	0.30	0.01	0.60

[1]Starch: effect of dietary starch concentration
[2]Grain: effect of barley grain, Dillon vs. Xena
[3]INT: interaction of dietary starch concentration and barley grain
[4]95% confidence interval; mean ± 2 × SD

Table 3. 5 The effects of dietary starch concentration and barley grain on total tract digestibility (%) of eight lactating dairy cows

	High Starch		Low Starch			*P* value		
Nutrient	Dillon	Xena	Dillon	Xena	SE	Starch[1]	BG[2]	INT[3]
DM	68.6	65.9	66.1	66.3	1.29	0.38	0.31	0.24
OM	70.5	67.9	67.9	68.3	1.29	0.37	0.39	0.23
Ether extract	76.1	73.1	71.1	71.4	2.04	0.12	0.52	0.43
CP	73.1	70.2	71.5	71.4	1.84	0.85	0.28	0.31
NDF	59.0	50.9	56.8	54.5	2.94	0.77	0.04	0.02
Starch	94.1	94.4	91.8	94.2	0.93	0.05	0.04	0.11

[1]Starch: effect of dietary starch concentration
[2]Grain: effect of barley grain, Dillon vs. Xena
[3]INT: interaction of dietary starch concentration and barley grain

Table 3. 6. The effects of dietary starch concentration and barley grain on digestibility of starch of four ruminally and duodenally cannulated lactating dairy cows

	High Starch		Low Starch			*P* value		
Item	Dillon	Xena	Dillon	Xena	SE	Starch[1]	BG[2]	INT[3]
Starch								
Intake, kg/d	6.19	6.79	4.71	5.20	0.275	< 0.001	< 0.001	0.46
Digested in the rumen								
kg/d	4.16	4.93	2.21	2.77	0.271	< 0.001	0.06	0.72
%	67.8	72.6	46.1	52.2	4.37	0.005	0.27	0.89
Passage to the duodenum, kg/d	2.03	1.87	2.50	2.41	0.269	0.05	0.54	0.87
Digested in the intestines								
kg/d	1.60	1.45	2.05	2.07	0.289	0.03	0.73	0.63
% of intake	25.2	21.1	43.7	40.6	4.23	0.004	0.37	0.90
% of duodenal passage	74.9	74.2	81.3	85.8	4.80	0.02	0.52	0.39
Digested in total tract								
kg/d	5.76	6.37	4.26	4.85	0.325	< 0.001	< 0.001	0.92
%	92.9	93.6	89.8	93.3	1.03	0.15	0.09	0.23

[1]Starch: effect of dietary starch concentration
[2]Grain: effect of barley grain, Dillon vs. Xena
[3]INT: interaction of dietary starch concentration and barley grain

Table 3. 7. The effects of dietary starch concentration and barley grain on digestibility of DM and OM of four ruminally and duodenally cannulated lactating dairy cows

Item	High Starch		Low Starch		SE	*P* value		
	Dillon	Xena	Dillon	Xena		Starch[1]	BG[2]	INT[3]
DM								
Intake, kg/d	20.7	20.9	20.3	20.7	0.94	0.31	0.28	0.72
Apparently digested in total tract								
kg/d	14.1	13.1	13.6	13.4	1.02	0.84	0.44	0.61
%	67.7	64.8	64.9	64.3	2.65	0.55	0.54	0.67
OM								
Intake, kg/d	19.3	18.8	19.3	19.1	0.871	0.64	0.30	0.70
Apparently digested in the rumen								
kg/d	4.61	5.58	5.87	5.33	0.546	0.18	0.53	0.06
%	24.8	30.4	30.2	26.9	3.16	0.46	0.54	0.03
Truly digested in the rumen								
kg/d	10.8	11.6	10.8	11.0	0.69	0.61	0.38	0.53
%	56.1	57.9	60.1	55.9	3.11	0.68	0.64	0.28
Passage to the duodenum, kg/d	13.7	13.3	13.4	14.8	1.11	0.25	0.38	0.13
Apparently digested in the intestines								
kg/d	7.86	7.02	6.99	7.25	0.822	0.60	0.64	0.35
% of intake	42.1	36.5	36.4	38.0	3.36	0.45	0.46	0.20
% of duodenal passage	55.0	50.5	51.8	55.4	3.56	0.80	0.90	0.28
Apparently digested in total tract								
kg/d	13.5	12.6	12.9	12.9	0.96	0.72	0.48	0.59
%	69.6	66.7	66.6	66.4	2.67	0.54	0.60	0.65

[1]Starch: effect of dietary starch concentration
[2]Grain: effect of barley grain, Dillon vs. Xena
[3]INT: interaction of dietary starch concentration and barley grain

Table 3. 8. The effects of dietary starch concentration and barley grain on digestibility of NDF of four ruminally and duodenally cannulated lactating dairy cows

Item	High Starch		Low Starch		SE	*P* value		
	Dillon	Xena	Dillon	Xena		Starch[1]	BG[2]	INT[3]
NDF								
Intake, kg/d	6.69	6.00	7.16	6.64	0.340	< 0.001	< 0.001	0.37
Digested in the rumen								
kg/d	1.45	1.36	2.36	1.61	0.345	0.07	0.15	0.25
%	21.3	24.3	32.7	23.9	6.28	0.22	0.49	0.19
Passage to the duodenum, kg/d	5.07	4.65	4.80	5.04	0.563	0.85	0.78	0.33
Digested in the intestines								
kg/d	2.12	2.81	1.54	2.00	0.725	0.63	0.88	0.39
% of intake	30.1	19.7	21.7	29.4	8.90	0.91	0.82	0.18
% of duodenal passage	42.7	30.9	32.1	33.6	9.55	0.44	0.33	0.22
Digested in total tract								
kg/d	3.8	3.17	3.9	3.59	0.504	0.49	0.17	0.57
%	56.4	52.5	54.4	53.3	4.52	0.91	0.60	0.76

[1]Starch: effect of dietary starch concentration
[2]Grain: effect of barley grain, Dillon vs. Xena
[3]INT: interaction of dietary starch concentration and barley grain

Table 3. 9. The effects of dietary starch concentration and barley grain on N metabolism of four ruminally and duodenally cannulated lactating dairy cows

	High Starch		Low Starch			*P* value		
	Dillon	Xena	Dillon	Xena	SE	Starch[1]	BG[2]	INT[3]
TRDOM,[4] kg/g	10.8	10.8	11.6	11.0	0.69	0.39	0.61	0.54
N Intake, g/d	627	594	620	620	23.5	0.34	0.14	0.12
Ammonia in the rumen, mg/dl	13.6	9.3	9.0	8.4	4.89	< 0.001	< 0.001	<0.001
Passage to the duodenum								
NAN[5]								
g/d	548	588	567	581	31.3	0.82	0.35	0.67
% of intake	87.6	98.5	91.8	94.9	5.16	0.94	0.15	0.40
NANMN[6]								
g/d	112	149	87	163	22.9	0.77	0.02	0.32
% intake	17.6	25.3	14.1	26.9	4.39	0.78	0.03	0.49
% of duodenal NAN	21.9	26.9	16.0	28.9	4.85	0.57	0.04	0.29
Microbial N								
g/d	426	438	480	418	48.2	0.67	0.55	0.37
% of duodenal NAN	78.1	73.1	84.0	71.1	4.85	0.58	0.04	0.29
g/kg of TRDOM	44.7	43.0	41.4	40.3	3.56	0.26	0.57	0.91
NAN digested in the intestines								
g/d	376	392	383	394	36.1	0.90	0.72	0.96
% of duodenal passage	68.2	66.9	70.1	67.4	3.53	0.76	0.61	0.86
N apparently digested in total tract								
g/d	455	398	436	433	29.4	0.69	0.16	0.20
%	72.3	67.2	70.3	69.3	3.41	0.92	0.30	0.42

[1]Starch: effect of dietary starch concentration
[2]Grain: effect of barley grain, Dillon vs. Xena
[3]INT: interaction of dietary starch concentration and barley grain
[4]TRDOM: true ruminally degraded organic matter
[5]NAN: nonammonia nitrogen
[6] NANMN: nonammonia and nonmicrobial nitrogen

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4.0. GENERAL DISCUSSION

Two experiments were conducted to evaluate the effect of two lots of barley cultivars, Dillon and Xena, on productivity and digestibility of lactating dairy cows. The first experiment used 31 lactating cows and its main focus was productivity. The second experiment used four ruminally cannulated cows and four ruminally and duodenally cannulated cows, and its main purpose was to evaluate ruminal fermentation and ruminal, intestinal and total tract digestibility for cows fed Xena and Dillon at two dietary starch concentrations. These studies demonstrated first that barley selection can affect milk yield and composition even though feed intake was similar. Secondly, the depression in milk fat concentration observed for cows fed Xena was likely due to the prolonged reduction in ruminal pH and higher insulin concentration, which are likely caused by a change in rate of starch degradation.

Previous research evaluating the effect of barley cultivars on animal productivity has mainly focused on swine and beef cattle and their findings are not consistent. Barley grain is variable in chemical and physical composition, and with over 50 barley cultivars grown only in Alberta, it is not cost efficient to test all cultivars, especially since grain composition is highly influenced by growing environment. Even though it is possible to analyze starch concentration of the grain, starch analysis alone does not always provide the entire picture. The amylose-to-amylopectin ratio in barley starch granule is also variable and since amylopectin has higher susceptibility to digestive enzymes than amylose, starch concentration per se may not be an accurate indicator of starch digestibility. Investigating the three-dimensional organization and location of amylose and amylopectin polymers could provide an insight on the physical and functional properties of starch.

It is important to gain an understanding on specific traits of the grain, such as nutrients, characteristics or their interaction, that affect the extent and rate of starch

degradability. This could be achieved by analyzing consistent correlations, for example correlations between NDF or CP concentrations and extent of starch degradation. Once these traits are identified, cultivars with desirable characteristics can be developed. Grain processing also influences the extent and rate of starch degradability. However, the extent of processing is expected to exert variable effects on productivity of dairy cows depending on quality of barley grain prior to processing, and therefore the optimal extent of barley grain processing deserves further experimentation.

APPENDIX

Appendix 1: NDF determination

1. Hot weigh dry, clean crucible (105°C)
2. Weigh 0.5 g dry, ground sample into a 600 ml beaker
3. Add 50 ml NDF solution and 75 µl amylase (Sigma A3306)
4. Turn on the water for the condensing unit, and turn on the switch for the burners
5. Place beakers on refluxing apparatus
6. At the onset of boiling add 0.5 g sodium sulfite to the beaker
7. Reflux for 1 hour
8. Slowly pour hot sample from the beaker into the crucible and filter solution using minimal vacuum
9. Rinse crucible with hot water
10. Fill crucible half full with hot water and 75 µl amylase
11. Let sit for 1 minute
12. Evacuate crucible and fill crucible with hot water
13. Let sit for 1-3 minutes
14. Evacuate and then fill crucible with hot water again
15. Let sit 3-5 minutes, repeat
16. Evacuate and then fill the crucible ¼ full with acetone and let sit for 3-5 minutes, repeat
17. Let residue and crucible dry for 60 minutes and place in oven at 105°C for at least 8 hours
18. Calculation:

%NDF = [(wt. cruc + residue) – (wt. cruc)] / (wt. sample) x 100

Appendix 2: Starch determination

1. Prepare acetate buffer 2 M, pH 4.9
    a. Dissolve 120 ml glacial acetic acid and 164 g sodium acetate abhydrous in 300-500 ml of d water in a 1000 ml volumetric flask. Dilute to volume
2. Determine free glucose in sample
    a. Weigh 0.50 g sample into 125 ml Erlenmeyer flask
    b. Add 50 ml dH2O to flask and swirl
    c. Allow the sample to hydrate 15 min
    d. Mix well and transfer ~5 ml sample to a polycarbonate centrifuge tube
    e. Spin at 26,000 x g for 10 minutes
    f. Decant 1.5 ml to micro-centrifuge tubes for analysis by plate reader
3. Starch gelatination and analysis
    a. Weigh sample into 125 ml Erlenmeyer flask. Be sure to include 100% starch standard (and known lab standard in each run)

<u>Following sample weight suggestions according to predicted starch %</u>:

0-10 %	0.50g
11-60%	0.25g
61-80%	0.20g
81-100%	0.10g

    b. Add 20 ml dH_2O to flask and swirl
    c. Allow the sample to hydrate 15 min

d. While swirling flask, add 0.5 ml 50% w/water NaOH and let sample gelatinize for 15 minutes. Consistently shake samples so that they do not concentrate in the middle of the flask

e. Add 10 ml 2 M acetate buffer to flask and rinse the sides of the flask with 15 ml dH_2O and swirl.

f. Add 0.8 ml concentrated HCl (12N) in a fume hood

g. Add 250 µl amylase (Crystalzyme 40L Valley Research, Inc., South Bend, IN) to each flask, seal each flask with aluminum foil and set in a 55°C water bath for 16 hours.

h. Transfer to a 200 ml volumetric flask and dilute with dH_2O to 200 ml.

i. Mix well and transfer ~5 ml sample to a polycarbonate centrifuge tube

j. Spin at 26,000 x g for 10 minutes.

k. Decant 1.5 ml to micro-centrifuge tubes for analysis by plate reader.

4. Glucose determination is the same as Appendix 6 except for different set of standards for free glucose determination (0, 2, 4, 6, 8, 10 mg Glucose/100 ml)

5. Calculation of results:

$$\text{Starch(\% DM)} = (0.9 \times [\text{Glc}]/1000 \times 2/W_s \times 100) - \text{Sugar \%}$$

$$\text{Sugar (\% DM)} = 0.9 \times [\text{Glc}]/1000 \times 0.5/ W_s \times 100$$

Where:

W_S = sample dry weight (mg)

[Glc] = concentration of glucose as read from the standard curve (mg/dl)

Appendix 3: INDF determination

1. Label ANKOM bags (Part #R510) with appropriate ID, use black permanent marker
2. Place bags in oven for 8 hours (105 °C)
3. Hot weigh bags
4. Weigh 0.5 g sample, and put into bag
5. Heat seal bags twice
6. Place bags into mesh bags
7. Place bags in cannulated cow for 120 hours
8. Remove mesh bags, place on ice, and wash mesh bags with cold water for 20 min (or until water is clear)
9. Weigh 2 g sodium sulfite into 600 ml beakers add 400 ml of NDF solution and place 15 ANKOM bags in the beaker
10. Turn on the water for the condensing unit, and turn on the switch for the burners
11. Reflux for 1 hour from onset of boiling
12. Rinse bags with water (~15 minutes)
13. Dry bags with sample for 8 h in 105 °C oven
14. Hot weigh bags
15. Calculate indigestible NDF

INDF% = [(wt. bag + residue) – (wt. bag)] / (wt. sample) x 100

Appendix 4: Crude fat determination

1. Weigh in duplicate 2 g dry, ground sample into a cone of an appropriate grade filter paper for the sample, then cover the material with small amount of glass wool
2. Weigh clean labeled extraction beakers
3. In a fume hood, add 40 ml petroleum ether (Fisher Scientific, Pittsburgh, PA, USA) into each beaker
4. Run at least one blank with 40 ml petroleum ether to determine residue after evaporation
5. Put samples in sample holders and attach to clamps at the condenser unit. Attach extraction beaker by tightening the beaker ring clamp
6. Turn on water condenser
7. Turn Goldfisch (Labconco, Kansas City, MO, USA) unit on and raise heater unit to near but not touching the beakers.
8. Once vigorous boiling has started check that there is no leakage of solvent
9. Extract fat for 4-6 hours with the heat set on high
10. Once extraction is completed lower the heaters and let the beaker contents cool
11. Remove each beaker so that the sample holder can be replaced with a glass solvent collector tube, then reattach the beaker
12. Heat until only a small amount of solvent is in the beaker
13. Lower heat unit again and turn off main power
14. Allow beakers to cool, remove them and transfer to a fume hood
15. Remove solvent collect and pour solvent into a waste ether bottle
16. Once all petroleum ether has evaporated place in a 110°C oven for 30 minutes

17. Cool beaker to run temperature in a desiccator and weigh

18. Calculation:

    % Fat = 100 x [(wt. beaker + wt. extract – wt. blank residue) – (wt. beaker)]

    / wt. sample

Appendix 5: Glucose assay

1. Dissolve the contents of 2 capsules of peroxidase-glucose oxidase (P7119, Sigma Chemical Co., St. Louis, MO) into 100 ml of d H_2O in an amber flask (solution A)
2. Using a squirt bottle, add 20 $\pm$ 0.01 g of d H_2O into the vial of dianisidine dihydrochloride (F5803, Sigma Chemical Co., St. Louis, MO; solution B)
3. Add 3.2 ml of solution B to 100 ml of solution A (solution AB). Solution can be stored at 4°C for 3 weeks
4. Prepare a standard glucose curve into test tubes using the stock solution in the glucose kit:

mg Glucose/100 ml:	*0*	*20*	*40*	*60*	*80*	*100*
Stock solution (µL);	0	200	400	600	800	1000
D H_2O (µL);	1000	800	600	400	200	0

1. Assay each sample in duplicate
2. Add 10 µL of sample, standard or blank (no sample) to wells in UV flat bottom 96-well plate (Corning Incorporated, Corning, NY, USA).
3. Add 300 µL solution AB to each well with a multi-channel micropipette, turn the plate reader on and shake the plate with the plate reader for at least 10 sec. Allow to sit at room temperature for 45 minutes covered with tin foil.
4. Read absorbance of samples at 450 nm

Appendix 6: Plasma insulin radioimmunoassay (Coat-A-Count ® Insulin)

1. Instructions are the same as provided in the kit, only difference is Calibrator B should also be diluted in half (B/2), for a total of 16 standards

Appendix 7: Non-esterified fatty acid enzyme immunoassay (NEFA-C Kit, Wako Chemicals USA, Richmond, VA)

1. Prepare buffer (50 mM phosphate buffer)
    a. 1M monobasic sodium phosphate (137.99 g/mol)
        i. Dissolve 69.0 g of monobasic sodium phosphate in 500 ml of water
    b. 1M dibasic sodium phosphate (141.96 g/mol)
        i. Dissolve 70.98 g of dibasic sodium phosphate in 500 ml of water (heat and stir to dissolve)
    c. Combine 5.77 ml of dibasic and 4.23 ml of monobasic sodium phosphate in a 100 ml volumetric flask and bring to volume. Check pH, should be 6.9-7.0
2. Prepare reagents
    a. Color Reagent A was diluted with 10 ml of color Reagent A diluent. Invert until dissolved then mix 13.3 ml of 50 mM phosphate buffer (pH 6.9). Solution can be stored at 4°C for up to 5 days.
    b. Color Reagent B was diluted with 20 ml of Color Reagent B diluent. Inverted until dissolved then mix with 33.3 ml of 50 mM phosphate buffer (pH 6.9). Solution can be stored at 4°C for up to 5 days
3. Prepare working standards (62.5, 125, 250, 500 µEq/L) by diluting stock standard solution with 50 mM phosphate buffer and stored at -20°C
4. Make standard curve

Conc of dilution = (Vol Std added/Total volume of dilution) * Conc of Std

5. Assay
    a. Assay each sample in duplicate.

b. Add 5 µL of sample, standard or blank (phosphate buffer) to wells in UV flat bottom 96-well plate (Corning Incorporated, Corning, NY, USA) in triplicate.

c. Immediately following add 95 µL of Color Reagent A and vortex.

d. Incubated at 21°C for 30 min.

e. Add 195 µL of Color Reagent B and vortex.

f. Incubated at 21°C for 30 min.

g. Read at 550 nm.

6. Calculations

From the regression output of the standard curve, formulate the line equation. Where Y is the absorbance reading, m is the slope, X is the NEFA concentration, and b is the intercept.

$$\text{Absorbance} = m(\text{NEFA } \mu\text{eq/l}) + b$$

$$\text{NEFA } \mu\text{eq/l} = (\text{Absorbance} - b)/m$$

Appendix 8: Ytterbium determination

1. Prepare 2% v/v nitric acid solution
    a. Add 20 ml concentrated HNO_3 to 980 ml double distilled water
    b. Dissolve 1.91 g KCl_2 into solution to act as an ionization buffer
2. Hot weigh empty, clean 100 ml beakers
3. Weigh 0.50 g of dried ground sample into hot weighed beakers in duplicate
4. Include two beakers as blanks
5. Dry samples in the oven at 135°C for 2 hours
6. Turn oven down to 105°C, wait 15 minutes and hot weigh beakers to obtain DM
7. Ash the samples in the beakers at 550°C for 5 hours
8. When samples are cooled to room temperature add 40 ml nitric acid solution to each beaker (to extract the Yb)
9. Place beaker on a shaker for 2 hours (100 rpm)
10. Transfer the solution in each beaker to a 50 ml centrifuge tube and centrifuge at 10,000 rpm for 10 minutes
11. Decant supernatant into labelled scintillation vial and analyze sample
12. Calculation

    Yb (μg/g DM) = (reading ppm x dilution factor) / (sample weight x DM)

Appendix 9: Rumen VFA assay

1. Sample preparation
    a. Thaw sample and pipette 5 ml into a centrifuge tube
    b. Centrifuge sample for 15 minutes at 3000 rpm
    c. Pipette 1 ml of centrifuged sample into a GC vial
    d. Add 200 µL of 25% phosphoric acid and 200 µL isocaproic acid solution (as an internal standard) and mix well
    e. Allow solution to stand for 30 minutes (if solution is not clear, centrifuge again at 3000 rpm for 15 minutes and analyze as soon as possible)
2. Internal standard
    a. 300 mg isocaproic acid in 100 ml distilled water
    b. Standard:

	g/100 ml water (+25% phosphoric acid to ph=3)	Approx. RF	Approx. mg/ml rumen fluid
Acetic	0.30	2.60	2-5
Propionic	0.20	1.60	0.5-2
Isobutyric	0.05	1.20	0.1
Butyric	0.10	1.20	0.5-1
Isovaleric	0.05	1.10	0.1
Valeric	0.05	1.10	0.1
Caproic	0.05	1.00	0.05

3. GC condition:
    a. Column: Stabilwax-DA 30 m x 0.25 mm I.D.
    b. Temperature: 120°C to 170°C at 10 °C per minute
4. Calculations:
    a. RF= response factor
    b. RF (acetic, A) = mg 'A' x area IS / mg IS x area 'A'
    c. mg 'A' / ml RuFl = (((mg IS / ml RuFl) x area 'A') / area IS) x RF 'A'

Appendix 10: Rumen and duodenal ammonia assay

1. Prepare reagents:

    a. Sodium Phenate

    12.5 g phenol + 6.25 g NaOH in a 500 ml volumetric flask, bring to volume with deionized water

    b. Sodium Nitroprusside

    Stock solution (1%)- 1g/100ml deionized water

    Working solution (0.01%)- 5 ml stock solution diluted to 500 ml (vol. flask) with deionized water. Working solution should be prepared daily

    c. Sodium Hypochloride

    0.02 N; 15 ml NaOCl (4-6%) diluted to 500 ml with deionized water. The pH should be adjusted to 12.0 with 50% NaOH

    All solutions should be stored in the fridge with tin foil wrapped around the containers and brought to room temperature before use.

2. Prepare standard solution: 100 μg NH_3-N/ml

    0.4176 g Ammonium sulfate (dried at 60°C for 2 h)/L deionized water- pH should be close to that of the samples

3. Sample preparation

    a. Rumen fluid

    - Thaw sample, mix well and centrifuge ~ 5 ml of sample for 10 min at 1000 x g

    - Pipette 20 μl aliquots into a 16 x 100 mm glass culture test tubes. Each sample is done in duplicate

    b. Duodenal liquid digesta

    - Thaw sample, mix well and centrifuge ~ 5 ml of sample for 10 min at 1000 x g

- Pipette 40 µl aliquots into a 16 x 100 mm glass culture test tubes. Each sample is done in duplicate

c. Duodenal solid digesta

- Weight ~3 g of sample into a 250 ml volumetric flask and bring to volume with water, let sample hydrate for 30 min

- centrifuge ~ 5 ml of sample for 10 min at 1000 x g

- Pipette 40 µl aliquots into a 16 x 100 mm glass culture test tubes. Each sample is done in duplicate

4. Color development

a. Prepare a standard curve by pipetting 0, 10, 20, 40, 50, 60 µl from standard solution in duplicate (further dilution may be necessary depending on NH_3 concentration of sample) into a 16 x 100 mm glass culture test tubes. Standards should be run in every set (~ 40 tubes). For "0" pipette 20 µl of distilled water

b. Add reagents to standards and samples in the following order:

2 ml Phenate solution and vortex

3 ml Nitroprusside and vortex

3 ml Hypochloride, cover tube with parafilm and invert several times

c. Develop color at room temperature in the dark for 1 h

d. Read absorbance at 600 nm using distilled water to zero spectrophotometer

Samples should be covered with parafilm at all times.

5. Calculations

a. Run regression using 0, 1, 2, 3, 4, 5 and 6 as x and absorbance as y

b. µg/volume added to tube = (absorbance – slope)/(slope)

c. Rumen fluid: µg/ml = µg/tube x 1000/(20 µl)

d. For duodenal digesta:

   i. NH_3-N (mg/ml) = (µg/volume added to tube)/(40 µl)

   ii. mg/250 ml = NH_3-N (mg/ml) x 250

   iii. NH_3-N (%DM) = mg/250 ml x sample weight x sample DM at 105°C